"Bayesian algorithm implementation in a real time calculating risk assessment model on benzene"

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Summary
The objective of the current study was the composition of a reliable modeling platform in order to calculate in real time the estimated health risk for filling station employees evaluating the current environmental parameters (traffic, meteorological and amount of fuel traded) determined by the proper sensors. A set of Artificial Neural Networks (ANNs) was developed to predict benzene exposure pattern for the filling station employees. Furthermore, a Physiology Based Pharmaco-Kinetic (PBPK) risk assessment model was developed in order to calculate the lifetime probability distribution of leukemia to the employees, fed by data obtained by the ANN model. Bayesian algorithm was involved in crucial points of both model sub compartments. The application was evaluated in two filling stations (one urban and one rural).

Among several algorithms implied for the development of the ANN exposure model, the Bayesian regularization provided the best results and in conclusion seemed to be a promising technique in the prediction of the exposure pattern of that occupational population group. On assessing the estimated leukemia risk under the scope of providing a distribution curve based on the exposure levels and the different susceptibility of the population, Bayesian algorithm was a completely necessary part of the Monte Carlo approach which is integrated in the PBPK model. Conclusively, the overall above modeling systems seems capable in exploiting the information gathered by the environmental sensors in order to calculate in real time the estimated risk of leukemia for the employees of the filling station.

Key words: Bayesian algorithm, ANN, PBPK, benzene exposure.

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1.Introduction.
Employees in fuel stations are exposed to high levels of gasoline vapours (including benzene) during refuelling as well as high concentrations of emissions from tail pipe exhausts. Therefore, service station employees are a vulnerable sector of the population because of their exposure during the 8h shifts [1], especially considering the well known health effects focused mainly on increased risk of leukaemia [2].

Until now, several remarkable studies were performed determining filling station employees personal exposure [3-5]. Most of the studies related to the employees exposure where limited to access the magnitude of exposure to benzene, usually in a shift time (8h duration) scale. Assuming that the dominant source of exposure is fuel evaporation, the study of exposure determinants was focused on empirical relations among the amount of fuel traded and ambient temperature and the obtained results where more qualitative than quantitative [6]. In the next years, after the adaptation of Stage II fuel evaporation recovery system in fuel pumps, exposure to benzene was significantly reduced (up to one order of magnitude). As a result, the effect of exposure parameters beside the refuelling procedure such as urban pollution levels and contribution of adjacent streets was more decisive, the quantification of the exposure determinants became a more complicated procedure.
The concept proposed in the present study is based on the concerns mentioned above. The overall aim is the development of a monitoring-modelling system able to evaluate in real time the continually altered values of the environmental parameters (detected by sensors) affecting the estimated risk would be of great interest. In order to achieve this, beside the sensoring of the environmental parameters, the development of the proper models-algorithms able to evaluate and process the acquired data is completely necessary. The main sub compartments of the modelling scheme are the exposure model which is an ANN model, which in turn feeds the risk assessment model, which is a PBPK-risk assessment model.

ANNs have been largely used to predict atmospheric pollutants concentrations, especially the reactive ones like NO\textsubscript{X} and O\textsubscript{3} [7], among several kind of other environmental applications [8]. The main reason for this is their remarkable ability to handle non linear systems. The non reactive pollutants are also non linear systems (in a much smaller degree than photoreacting pollutants) since they are highly depend on atmospheric dispersion conditions and vehicles emissions. Recently, non reactive pollutants forecasting via ANNs [9,10], has gained an increased interest, because ANNs can handle the large amounts of data, now routinely acquired through modern monitoring techniques but according to our knowledge, ANNs have not been used so far for the prediction of human exposure. In the present study, the developed set of ANNs is aiming to produce an exposure predicting model, quantifying also the contribution of the parameters that constitute the exposure pattern.

As a final step after the determination of exposure and the related parameters, the potent health implications were assessed through a developed PBPK/PD model. The concept of the model is the determination of concentrations of benzene metabolites (catehol, phenols, muconic acid, phenylmercapturic acid and hydroquinone) on target issues (bone marrow) and based on the dose response curve to calculate the estimated risk, considering also the diversity of the population through a Monte Carlo-Markov Chain approach. PBPK models are a very recent technique on risk assessment [11] providing biology based approach which is more rational and sophisticated for estimating potential health implications than empirical relations extrapolated from epidemiological studies.

2. Methodology.
2.1. General
The aim of the study is the development of a platform which:
- Gathers information from crucial environmental parameters (through the proper environmental sensors) which constitute determinants of the exposure to benzene of filling employees
- Implements the proper algorithms evaluating the information obtained by the environmental sensors, in order to provide real time estimation (a) of exposure to benzene and consequently (b) of the associated health risk.

As a result, the main interest of the present study lies on the development of the modelling schemes that utilize the information gathered by the sensors. For this purpose, two individual modelling concepts where adopted, one for the calculation of exposure to benzene which is an ANN based model and one for the risk estimation due to exposure to benzene which is a PBPK model. A visual presentation of the whole platform is presented in Figure 1.

Beside the theoretical complexity of the study, it is important to be clarified that both modelling compartments where developed-evaluated based on the results of real experimental procedures, including environmental field measurements (ANN model) as much as laboratory studies (PBPK model). Thus, the field measurements campaign constitutes a significant part of the whole study and the adopted techniques, as much the related results are extensively described.

2.1.1. Field measurements campaign design
The campaign has taken place in Ioannina, a medium sized (nearly 100,000 inhabitants) city in North-Western Greece, with relatively high values of air pollution [12]. Given that near the wider area no significant industrial activity is observed, the dominant source of ambient air pollution is traffic, contributed by central heating during winter. The measurements campaign was performed during two sampling periods (August and December, 2006) to investigate the seasonal variation of benzene exposure values. The study was performed at two filling stations, an urban one and a rural one, at a distance of 15 km of the city limits. Both of them were equipped with Stage II fuel evaporation recovery systems. The location of the stations and the adjacent roads, in relation with the prevailing wind direction are described in Figure 2.

Personal exposure to benzene by the method of passive sampling was performed to 15 volunteers each season for two weeks during the working hours (8:00-16:00). One sampler per day for each volunteer was used. The
volunteers group included 10 employees in the urban filling station and 5 in the rural one. People working in
filling stations were doing three major activities: cars refueling, miscellaneous activities (like car washing or oil
checking) and cash machine occupying in the filling station office. Most of the employees were doing a
combination of these activities, especially in the rural filling station.

In order to examine the daily exposure variation and the significance of the activities in the configuration of the
total amount of exposure for the filling station employees, active sampling was also performed. In this case,
sampling pumps were attached on the employees, and the samplers were placed in the height of the chest, just
like the commonly known passive samplers. This was attainable due to the relatively small size and weight of
the sampling pumps. The employees carrying the pumps were executing strictly only one kind of activity and
consequently were employees refueling cars, miscellaneous activities employees or cashiers. The pump was
setting to work for 30 minutes in a flow rate of 100 ml/min. Each sample was attached for half hour, and one
sample was used per hour, so until the end of the 8h shift, 8 samples per employee were taken. In each filling
station, one employee of each category was carrying the active sampler. Active sampling was performed day
day. Totally, 224 samples were taken.

Apart from active sampling, the following parameters were measured, and all the values were recorded in a 30’
basis:
- The amount of the gasoline that was traded.
- Wind speed (m/sec) and direction (degrees).
- Ambient air temperature.
- Traffic flow from two independent observers, where the vehicles were registered in seven main categories
  (catalytic passenger vehicles, non catalytic passenger vehicles, diesel passenger vehicles, light duty
  passenger vehicles, heavy duty passenger vehicles, buses and motorcycles) every half an hour. In the urban
  site the measurements were continuous from 8:00 to 16:00. Vehicles speed was calculated by the quotient of
  the distance (a part of the road) and the time, which was needed to cover that distance.
- Urban background concentration. The commonest (and more reliable) modelling practice to define
  background concentration is to use data obtained from measurements at urban locations that are not
directly affected by local sources [13]. Based on this, two active samplers were placed in two different urban
  locations (not affected by traffic or other known benzene sources) and their values were averaged in order
  to exclude the urban background concentration. In the rural area also two passive samplers were placed to
  investigate any possible background concentration or the existence of any other benzene source beside the
  road and the gas station emissions that may affect the measurements results. In the operational mode of a
  multi-sensor fusion based monitoring system as the one outlined in this study, these parameters would be
  measured using automated sensors and their output data streams integrated following exactly the same
  algorithm as the one given in this paper.

2.1.2. Instruments and apparatus
The equipment used during the measurement campaign and analyses is listed below:
1. Gas chromatography system Varian 3900 GC with flame ionization detector (FID). The capillary column
   through which the chromatographic separation of the various pollutants was effected, is the 30 m long, 0,53
   mm inner diameter and 0,5 μm film thickness, SBM™_5 capillary column, by Supelco, Italy.
2. Thermal desorption system MARKES Thermal Desorption Cold Trap Injector
3. 3 low volume pumps for gas sampling SKC model 222
4. Flow meter with a measurement range of 0,010 to 12 lt/min, DryCal CD-Lite (Bios International, USA)
5. Sampling tubes (proper both for active and passive sampling) MARKES CARBOGRAPH 1TD tubes
   standard absorbing cartridges filled with 400 mg of sorbent.
6. Cup anemometer and wind vane Qualimetrics, model 2020 and 2032, respectively.

2.1.3. Quality Assurance and Quality Control
The quality assurance and quality control (QA/QC) procedure included laboratory and field blanks, parallel
samples and duplicate measurements of samples. Before each sampling with a canister, blank sample (zero air)
was analyzed to ensure that the concentration of all compounds inside was below 0.02 ppbv. Parallel samples
and duplicate measurements of samples were analyzed to test the precision of the sampling and analytical
techniques, respectively. The mean relative standard deviations (RSD) for all the compounds were less than 8%.
A new calibration curve was determined each time. The detection limit of each compound was calculated from
the data of seven replicate measurements of low concentration samples and observed from their standard
deviation. Correlation coefficient for benzene was 0.9983. Response linearity tests showed that the response signal was proportional to injection volume for benzene concentrations (0.5–220 μg/m³).

2.2. ANN (Artificial Neural Network) modeling development.

Intending to obtain a way to predict the exposure pattern of the filling station employees, the development of a set of ANNs was introduced. The main concept was to use as input parameters (i) the amount of gasoline sold, (ii) the traffic flow of the road in front of the filling station, (iii) the background concentration (only in the urban filling station because in the rural it was almost zero), (iv) the wind speed, and (v) the ambient temperature, in order to predict as output the exposure pattern of the three main subcategories of employees mentioned above. Consequently, three ANNs were employed, one for each group of workers. Furthermore, the ANNs can provide significant information for environmental policy purposes, after quantifying the parameters that constitute the exposure pattern, in terms of the Relative Importance (% contribution values).

In their general form, ANNs refer to a parallel model architecture able to perform numerical calculations based on distributed processing. ANNs are composed from artificial neurons that operate according to a specified transfer function. The neurons interact with each other through the use of neuron connections called weight factors or simply weights. Positive or negative weights correspond to connections that propagate or suspend, respectively, signals from other neurons. ANNs can be trained to perform a particular function by adjusting properly the values of these weights.

In this approach a feedforward network architecture was developed, which is widely used in ANN applications. This is a multilayer architecture, meaning that the artificial neurons are organized in layers. Typically, a standard feedforward ANN has an input layer, where data are introduced to the ANN, one or more hidden layers, where processing is realised, and an output layer, which generates the final results (Fig. 3).

Another important characteristic of feedforward ANNs is that neuron activation (or signalling) propagates towards one direction only and specifically from the input layer to the hidden layers and finally to the output layer.

In this study, 3 different sets of ANNs were developed: one for the urban station, one for the rural and one for both. The number of the neurons corresponds to each input parameter. The input parameters were the amount of gasoline traded, wind speed, ambient temperature and traffic flow of the road in front of the filling station and benzene urban background concentration. The difference between the first and the other two sets of ANNs are that in this case, the first (input) layer consisted of 5 neurons while for the rural one consisted of 4, due to the lack of background concentration. In the third set, data gained from both stations were used for training and testing. In this case, the lack of the background concentration as an input parameter was not so determinant for the urban stations’ predictions, because it is indirectly included in the parameters of traffic flow and wind speed. We should note that in all cases the ANNs’ architectures were kept the same. Specifically, we met in the input layer four or five nodes, in the hidden layer 10 and in the output one (Fig. 3).

In particular, the second (hidden) layer consists of 10 neurons that implement the hyperbolic tangent sigmoid transfer function. This function generates outputs between -1 and 1 as the neuron’s net input goes from negative to positive infinity and is more appropriate for ANN applications on function approximation. It should also be noted that the input parameter values are pre-processed in order to be normalized according to Eq. 1, the inputs and targets will fall in the interval [-1, 1].

\[
x_n = 2 \frac{x - \min(x)}{\max(x) - \min(x)} - 1
\]

As for the number of the hidden neurons, various architectures were tested (different numbers of neurons and hidden layers) but the choice of one hidden layer with 10 neurons provided consistently the best results. Finally, the third (output) layer consists of three linear neurons that correspond to the three predicted exposure concentrations for each one for each exposed group of workers.

Network training was performed using the Bayesian regularisation algorithm [14-15]. This is a supervised learning method that determines the optimal network parameters in terms of model generalisation. These parameters, namely the weights and biases of the network, are assumed to be random variables with specified distributions. The (regularization) parameters are related to the unknown variances associated with these distributions. Consequently, based on statistical techniques these parameters can then be estimated.

Within this framework the following error function is minimised:
\[ E = a_1 \sum_{i=1}^{N} (t_i - o_i)^2 + a_2 \sum_{i=1}^{M} w_i^2 \]  

(2)

where \( t_i \) are the desired network outputs, \( o_i \) are the network outputs during training, \( w_i \) are the network parameters (weights and biases), \( M \) is the number of those parameters and \( N \) is the number of the training patterns. The hyperparameters \( a_i \) are estimated at each iteration according to the following formulas:

\[
a_1 = \frac{N - \gamma}{2 \sum_{i=1}^{N} (t_i - o_i)^2}
\]

(3)

\[
a_2 = \frac{\gamma}{2 \sum_{i=1}^{M} w_i^2}
\]

(4)

where \( \gamma \) is the number of effective parameters and is given by:

\[
\gamma = N - 2a_2 \text{tr}(H)^{-1}
\]

(5)

with \( H \) being the Hessian matrix of the error function and can be approximated as follows using the Jacobian matrix, \( J \), that contains the first derivatives of the network errors with respect to the weights and biases:

\[
H = J^T J
\]

(6)

The network parameters \( w_i \) are updated according to the Levenberg-Marquardt optimisation method:

\[
w_{i+1} = w_i - \left[ J^T J + \mu I \right]^{-1} g
\]

(7)

where \( I \) is the unit matrix, \( \mu \) is a scalar parameter \([16]\) and \( g \) is the gradient which can be computed as:

\[
g = J^T e
\]

(8)

with \( e \) being the vector of network errors.

The network parameters are initialised using the Nguyen-Widrow method \([17]\) and the two hyperparameters, \( a_1 \) and \( a_2 \), are initially set to one and zero, respectively. Apparently, the error function \( E \) is adapted at each iteration, since the hyperparameters \( a_i \) are re-estimated. According to the Bayesian regularization algorithm the training procedure is considered to be completed when the effective number of parameters, \( \gamma \), has converged, indicating that the ANN has generalised and not overfitted the training data.

Besides Bayesian regularization, the performance of the proposed methodology using various training algorithms on different network architectures was also evaluated. Specifically, algorithms from simple variants of the well known Backpropagation like the Resilient Backpropagation \([18]\) and the Scaled Conjugate Gradient \([19]\) to more sophisticated ones like the BFGS \([20]\) and the Levenberg-Marquardt \([16]\) were employed.

For training and testing the proposed ANN model we followed the procedure of Leave-One-Out Cross-Validation (LOOCV). According to this schema, iteratively one sample is kept for testing while the rest are used for training the model, until all samples are finally tested \([21]\). There are many ways to implement cross-validation, however LOOCV is a very commonly used validation schema and is considered to better resemble real practice where the proposed model is trained with all the samples at hand and then is applied to the specific application.
2.3. PBPK risk assessment model

Physiologically based pharmacokinetic (PBPK) models propose a realistic even if simplified description of the mechanisms of absorption, distribution, metabolism and elimination of chemicals in the body. In these models, the body is subdivided into various compartments representing specific organs or homogeneous groups of tissues linked and irrigated by blood vessels. Compartments are characterised by a set of parameters of physiological relevance (e.g., volume or blood perfusion rate) which play a crucial role in explaining the behaviour of chemical substances in the body, and represent invariants across substances. Physiologically based pharmacokinetic (PBPK) models offer great flexibility. In particular, they provide a parametric framework suitable for dealing with extrapolations between species, routes or dose levels [11, 22-23].

The PBPK model for a generic mixture of chemicals is represented as a combination of “single chemical” models interconnected at level of hepatic metabolism where the effect of the interaction is evaluated according to the potential mechanism of action (competitive, noncompetitive, and uncompetitive metabolic inhibitions). The latter, in the case of a BTEX mixture, is assumed to be of “competitive inhibition” since the four VOC’s considered are known substrates for the same cytochrome P450 isozyme (CYP2E1). This was confirmed by the analysis of the kinetic data from all binary exposure studies relevant to the BTEX mixture [24]. The models for toluene, ethylbenzene and all the family of xylenes are all four-compartment models encompassing richly perfused tissues (RPT), poorly perfused tissues (PPT), adipose tissues (FAT), and liver (metabolising tissue), interconnected by systemic circulation and a gas exchange lung.

The model for benzene is a six-compartment model. The six tissue groups include: liver (main metabolic tissue); adipose tissue (FAT); richly perfused tissues (RTP); poorly perfused tissues (PPT); bone marrow (the main target organ for benzene toxicity) and the kidney; each one interconnected to the others by systemic circulation and a gas-exchange lung. The bone marrow was included because it is recognised as the main site manifesting benzene toxicity (i.e., leukaemia) and because it is, together with the kidney, a potential site benzene metabolism. The liver was further subdivided into three equal volume sub-compartments according to the zonal distribution of enzymes that mediate benzene metabolism [25]. Metabolism mediated by CYP2E1 is assumed to occur by and large in “zone 3” of the liver; sulfation takes place primarily in “zone 1”; non-enzymatic metabolism occurs in all the three compartments as well as in all tissues.

The high potential toxicity of benzene metabolites associated to the leukaemia risk in humans, suggested taking into account more in detail the metabolic chain from benzene to its key metabolites through a more refined PBPK model for that chemical. The whole metabolic chain of benzene was modelled starting from previously developed PBPK models for benzene metabolism in mice [25] and its extrapolation to humans [26], which in turn where based on experimental data of individuals exposed to benzene. The model (Figure 3) 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 produced. For benzene oxide, phenol, and hydroquinone, the body is divided into five compartments: kidney; liver; fat; rapidly perfused tissues (RTP), and slowly perfused tissues (PPT). As for the benzene model the liver is subdivided into three compartments of equal volume according to the specific enzymatic distribution. The further metabolism of BO, PH and HQ is supposed to occur in the liver (main metabolism organ) and to a lesser extent in the kidney.

More in detail the followings metabolic transformations (mediated by CYP2E1) are supposed to occur in zone 3 of the liver as well as in the kidney:

- Benzene → Benzene oxide
- Phenol → Hydroquinone
- Phenol → Catechol
- Hydroquinone → Trihydroxy benzene

Since the kidney contains approximately 10% of the concentration of CYP2E1 found in the liver [27], it is assumed that relative to the metabolism in the liver, 10% of the metabolism mediated by CYP2E1 is in the kidney.

Having in mind that cancer risk associated to benzene is mainly related to its metabolites 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, starting from prior distributions of the most important physiological and biochemical parameters influencing the determination of the internal dose of benzene metabolites we can derive a distribution function of health risk to the exposed population rather than a unique value representing an “individual” risk using Markov Chain Monte Carlo techniques [28]. In particular, starting from literature-
obtained prior distributions of key biochemical and physiological parameters known to affect the level of individual susceptibility to xenobiotic health stressors such as age, gender, weight, fat percentage and distribution in the human body. We can use Bayesian statistics to calculate posterior distributions of the same parameters in an iterative manner. Thus a Markov chain of Monte Carlo simulations is developed until the resulting posterior distributions converge to the actual parameter distributions used for estimating the internal and biologically effective dose of the benzene metabolites at the target organs (e.g. the bone marrow) and, consequently, the related cancer pathology risk. In this way, the estimated cancer risk is representative not only of an average individual as in the case of most health risk estimates used to date; instead, the resulting risk outcome is expressed in terms of a probability distribution of cancer risk as a function of the population size and the exposure levels. Individual risk is transformed into the corresponding population risk providing thus a better metric of public health pressure associated to environmental exposure to benzene and its metabolites under realistic and real-time conditions. The whole PBPK/PD and pathology (risk) model was developed on acsXtreme simulation software.

3. Results and Discussion.

3.1. Traffic data
As it was mentioned before, traffic flow and fleet composition (Table 1) on the roads adjacent to the filling stations was measured and traffic speed was calculated. The fleet pattern from the urban station road is dominated by passenger cars equipped with a catalytic converter (70% of the total fleet). The percentage of passenger cars not equipped with a catalytic converter is also significant (8%), followed by motorcycles (7%). Both of them present strong benzene emission potency. In the rural road the fraction of heavy diesel vehicles (trucks and buses) is significantly increased (14%).

Traffic flow in the urban adjacent road presents an average flow of 1500 veh/h, while for the rural road is about 600 veh/h. In the first case, traffic flow presents maximum at 8:00-9:00 and at 2:00-3:00 related to the transportation of citizens to working places. In the rural road, traffic flow seems to be more uniform, presenting a slight increase at 10:00-11:00. Daily variation of traffic flow is presented in Figure 3, where the averaged weekly variation is shown.

No significant seasonal variability was observed in the urban street, while in the rural road, traffic flow was increased almost to 20% in summer time. Traffic speed in both cases was calculated to be equal to the road limit (50 and 70 km/h respectively), having a deviation of ±10 km/h.

Traffic data are necessary to estimate the magnitude of benzene’s road emission rate and consequently to have an overview of the importance of these emissions to the employees exposure pattern. Based on the daily traffic variation data, benzene emission rate for urban and rural road were calculated, varying from 1.4 - 0.26 gr.km\(^{-1}\).sec\(^{-1}\) and 0.5 - 0.11 gr.km\(^{-1}\).sec\(^{-1}\) respectively by using COPERT III methodology [29].

3.2. Meteorological data
Wind speed and direction measurements confirmed the historical data on the wind field over the area. The prevailing wind directions, during the sampling periods, were from southwest and northwest directions and are presented in the location maps (Fig.2).

In the urban area, mean wind speed ranged from 0 to 7 m/sec, for the summer period, while the average wind speed was 2 m/sec. In summer, wind speed tended to increase after the evening hours. In the winter, wind speed ranged from 0 to 5 m/sec, having an average value of 1.1 m/sec. In the rural location, wind speed had the same profile, but the mean values where in general increased by a factor 20-30%.

Ambient temperature in the urban area ranged from 14 to 28 °C, having an average value of 23 °C for summer, while for the winter the corresponding values are -2 to 13 °C and 7 °C respectively. For the rural filling station the values (min-max-average) for summer and winter were 11-26-22 °C and -4-12-6 °C respectively. Temperature variation through the day was in general the classical, increasing from the morning to the evening.

3.3. Amount of gasoline traded
The amount of the gasoline traded during the working hours was recorded automatically by the cash machines which are wired to the fuel pumps in both filling stations. In the urban filling station, the amount of gasoline ranged from 550 to 950 l/h, having an average value of 700 l/h, while for the rural station ranged from 150 to 300 l/h, having an average value of 200 l/h. Daily and seasonal variation of the gasoline amount was proportional to the adjacent road traffic flow variation. The averaged weekly daily variation of urban filling station is also presented in Figure 5.
3.4. Personal exposure results

Exposure pattern for the filling station employees is complex, presenting significant variation of the recorded values. In Figure 6 the weekly averaged results of passive sampling are presented. The values ranged from 51 to 18,3 $\mu$g/m$^3$ in summer and from 41 to 17,5 $\mu$g/m$^3$ in the winter for the urban filling station. In the rural filling station, the values range from 29,5 to 10,3 $\mu$g/m$^3$ and 26,8 to 9,1 $\mu$g/m$^3$ for summer and winter respectively. From a recent exposure survey conducted in the same urban area [30], exposure to benzene for the general population was determined equal to 9 $\mu$g/m$^3$, thus exposure to benzene for the filling station employees is considered elevated, although the presence of the preventing measures by Stage II fuel evaporation recovery systems adopted.

The higher exposure levels in the urban filling station may be explained by the total amount of gasoline daily distributed (6000-12000 liters of gasoline in comparison to 2000-3000 liters) and to the absence of any other significant source of benzene in the rural filling station. On the contrary, the emissions from the central road in front and the contribution of the urban background concentration in the urban filling station seem to be significant additive parameters.

A seasonal variation was observed, especially in the urban filling station. The increased benzene concentrations in personal exposure values, while also in the vicinity of the filling station are explained by the higher temperatures occurring in summer (average temperature 23 °C, while in winter is 7 °C), due to stronger fuel evaporation. In the urban filling station this observation is more intense, due to the bigger amount of gasoline distributed (and consequently evaporated). Furthermore, the corresponding increase in background concentration and direct traffic emissions from the adjacent road, concurrent to the recorded variation among summer and winter measurements.

Results of active sampling measurements were necessary to clarify the importance among the activities occurring in a filling station. In Figures 7a-b, the hourly averaged weekly active sampling measurements results for each category of employees are presented.

From the results it is understood that employees refuelling cars present the higher exposure levels in benzene. This was an expected result because the dominant source of benzene is car refuelling. Although that newer fuel pumps with automatic fuel counters do not demand the presence of the employee above the fuel hose during the refuelling, the fact they resident or move closely to the pumps wider area where the concentrations are high, leads to the recorded elevated exposure values.

Daily variation of exposure values of employees working outdoor, seemed to be highly correlated to the amount of gasoline distributed, an observation confirming the results from older studies [6]. Gasoline trading rate is higher in the morning (8-9:00) and evening hours (14-15:00), related to the transportation of citizens to working places. This incident is not clearly observed in the rural filling station, were only in the morning an increase of the distributed gasoline is noticed. Because the variation of fuel traded is in linked up to traffic flow, maybe by mistake someone overestimate or underestimate the importance of traffic flow in the configuration of the exposure pattern. It is important to clarify in this moment of the study, that the increased traffic flow of the adjacent road leads more customers to visit the filling station and consequently the increase in fuel traded.

Ambient air temperature also affects significantly the exposure levels. When temperatures are near 0 °C (a fact occurring at the early morning hours in the winter time), the exposure levels are lower than usual for the same amount of fuel traded (Figure 7).

The presence of wind seems to reduce exposure levels, especially to miscellaneous activities employees. No strong correlation among wind speed and exposure levels of employees refueling cars and cashiers was observed.

Finally, in the urban station, background concentration seems well correlated to the exposure levels of all the categories of employees, but the reason of that correlation is the same with the traffic flow, because when the traffic flow is raised, this occurs in the whole urban area and consequently the urban air pollution is elevated. The quantification of the importance of the above parameters is further investigated next through the ANNs modeling.

Compared to employees refueling cars (Figures 7a-b), employees of miscellaneous activities are exposed to significantly lower benzene concentrations, because they rarely go near to the pumps, staying mainly in the area of car washing machines. This means that they work in an average distance of 15-25 meters from the pumps, where the concentrations of benzene are significantly reduced.

The cashiers are working in an indoor environment, which is also affected by the same parameters that configure concentrations in the wider vicinity. Thus, the recorded values are lower to the employees of
miscellaneous activities. The only remarkable difference at the rural filling station (in comparison to the urban one) is the absence of any significant variation among summer and winter exposure measurements of the cashiers. This maybe occurs due to an additive indoor source of benzene affecting the concentrations, possibly smoking, which is permitted inside the filling station office.

3.5. Artificial Neural Network modelling results
The results among the observed and the predicted values are shown in Figures 8a-b. The evaluation parameters RRMSE (Relative Root Mean Square Error) and $R^2$ (Correlation) are presented in Table 2. From the above, it is easily observed that the performance of the ANN applied to the urban filling station data is slightly better than the ANN applied to the rural one for the first two categories of employees. On the contrary, the performance referring to cashiers is worst in the urban station. This occurs because the indoor concentration of benzene presents a time lag following the outdoor concentrations that determine the exposure of the other two categories of working people and is strongly affected by the general benzene concentrations that affect the wider area.

This is also the reason, that among the three categories of employees, the best performance of the model is for the miscellaneous activities employees. Exposure pattern of these employees is directly affected by the referred input parameters, located in the area of the station that does not appear to have acute changes in the observed benzene concentrations. On the contrary, this occurs to the employees refueling cars during the refueling process, having as a consequence sometimes the quickly elevated levels of exposure to be hardly predicted with the same degree of accuracy.

The results of the third ANN are also very good, presenting similar evaluation values. This reveals that the model in its general form is very well fitted in filling stations with different characteristics of the emission sources. Having in mind the simplicity of the model (one input parameter fewer) and the fact that for both the filling stations one model is required, for applications aiming to predict the exposure levels of the employees, this is the intended model.

3.6. Relative importance of the parameters constituting the exposure pattern obtained by the ANN model
The main reason to apply two sets of ANNs (for each filling station) comes from the exigency to quantify the relative importance of the parameters that determine the exposure levels. Network modelling can assess the importance of each of the input variables by using the network weights. The method for partitioning the connection weights proposed by Garson [31] was used. The technique involves partitioning the hidden–output connection weights of each neuron into components associated with each input neuron [32]. The results of the calculations are shown in Figures 9a-b, displayed as columns representing the relative importance of the various input variables.

These results in combination with the active sampling ones clarify the relations of the emission sources (car refuelling and the corresponding amount of gasoline traded, direct traffic emissions and background concentration) with the meteorological parameters (wind speed and temperature). In both filling stations, the amount of the fuel traded is the dominant parameter of exposure for all the categories of employees. The importance of that parameter is decreasing, in a way proportional to the distance of the fuel pumps an employee is occupying, thus this parameter is more important for employees refuelling cars, especially them working in the urban station, were the fuel amounts are higher.

Wind speed is also an affecting parameter for the employees working outdoor. This is explained by the fact that the employees working outdoor are directly exposed to wind which affect influential the ambient benzene concentrations. The importance of the wind is stronger in the rural filling station, because the dominant source emitting benzene (car refuelling) is not so strong like in the urban station and the ambient concentrations are more affected to dispersion parameters.

Temperature is a determinant parameter, affecting fuel evaporation and consequently the presence of benzene in air. The effect of temperature in both filling stations is stronger than winds’ effect, not only based on the daily variation, but also based in the average exposure values, that in summer are in general elevated. An exception of this acceptance occurs for the employees refuelling cars in the rural filling station.

Traffic flow importance of the road adjacent to the filling stations, has a smaller effect than the other parameters, because the dominant source of benzene in the filling stations has local origin. Some differences are observed related to the distance of the road that the employees are located. Because urban station is larger, employees occupying miscellaneous activities are located in greater distance from the adjacent road than these from the rural one. On the contrary, the emission rate from the urban station adjacent road is significantly
higher, resulting in the increased importance compared to the rural one. In the rural filling station, this parameter seems to have a significant effect for the cashiers, which is practically impossible. In this parameter the network probably include all the unknown parameters that determine the exposure pattern of that category of employees.

Finally, urban background concentration appears to have a small effect in the configuration of the exposure values, affecting mainly the cashiers. Background concentration express the ambient benzene levels in the wider area, and this value present a slow response to the parameters affecting ambient benzene levels in a way similar to the exposure of cashiers to the exposure parameters. This parameter, as mentioned above, could be excluded from the network for reasons of simplicity, without decisive loss in the accuracy of the predictions, but if the model will be applied in a filling station located in a more polluted area, is necessary to be included.

3.7. Potential health risk estimation

Due to the fact that measured benzene concentrations and the related exposure levels do not constitute a danger for acute effects, the main concern is related to the possibility of leukemia incidences. The estimated risk due to benzene exposure of general population in the area under study is calculated considering:

- The exposure to benzene values obtained by the measurement campaign
- The related concentrations of benzene metabolites on target issue (bone marrow) through the developed PBPK/PD model and the related dose-response curve
- The diversity of the population through a Monte Carlo-Markov Chain approach

The whole concept in developing a PBPK model is the use of a dynamic model for risk estimation, able to calculate possible interactions among the levels of benzene exposure through different activity patterns that may possibly lead to completely different concentrations of benzene and of the related metabolites in the blood and consequently to misjudged risk estimations. Furthermore, the model has the ability to calculate also the effects of toluene, xylene and ethylbenzene to the metabolism of benzene. These compounds compete with benzene for CYP2E1, thus these co-exposures significantly reduce the overall metabolism of benzene relative to equivalent exposures of pure benzene. At low levels of exposure, competition for the P450 binding sites would be minimal and the same occur in the reduction of benzene’s toxicity due to co-exposures. In the present study, both interactions mentioned above were extensively examined and under the exposure levels determined, no significant interactions were observed.

The results of the calculations are presented in the possibility distribution plot of Figure 10. The obtained distributions represent the variation on the activity patterns among the several individuals which contribute to different levels of exposure and the diversity of the population which is reflected as a different degree of response to environmental stressors. Benzene’s toxicity is intimately tied to its complex metabolism and distribution. Key enzymes involved in the metabolism of benzene, including CYP2E1, the quinone reductase NQO1, and myeloperoxidase are polymorphic. For example, a 7.6-fold difference in benzene-induced hematotoxicity in workers was observed among gene variants of CYP2E1 and NQO1. Thus the expression of genetic polymorphisms may modulate the sensitivity of an individual or ethnic group to the effects of benzene exposure.

The average estimated risk for the general population based considering exposure to benzene equal to 9 μg/m^3 [30] is equal to 4.7·10^{-5}, ranging from 2.4·10^{-5} to 9.4·10^{-5}. For the filling station employees, risk calculation is slightly more complicated, because the parameter of occupational exposure \( C_o \) has to be inserted in the calculations. Having in mind that the people work for 30 years, for 5 days a week and 8 hours a day, and for the rest of their lifespan are exposed to the same concentrations \( C_c \) of the control group, the average exposure \( C_A \) for a 70 years life span is calculated through the equation:

\[
C_A = C_o \cdot \frac{30y \cdot 5d \cdot 8h}{70y \cdot 7d \cdot 24h} + C_c \left( \frac{40y \cdot 30y \cdot 2d}{70y \cdot 7d} + \frac{30y \cdot 5d \cdot 16h}{70y \cdot 7d \cdot 24h} \right)
\]

According to the above, the estimated risk for the occupational groups (as an average, minimum and maximum) for the urban gasoline station employees was estimated equal to 5.7·10^{-5}, 3.1·10^{-5} and 11.2·10^{-5} (Figure 10). For the rural gasoline station, the related risk is slightly lower and the corresponding values are equal to 5.2·10^{-5}, 2.7·10^{-5} and 10.1·10^{-5}. It also needs to be clarified that this distribution of leukemia possibility is based on the average exposure levels of exposure for the employees of both filling stations. In case that the employees are continuously performing one kind of the three main activities, then three new distributions are obtained (Figure 11), indicating the
increased risk for the people dealing with car refueling ($7.0 \times 10^{-5}$, $3.4 \times 10^{-5}$ and $13.3 \times 10^{-5}$ the corresponding values of the urban filling station employees as an example).

Although the use of benzene is being progressively reduced over the last ten years, exposure to benzene still constitutes an environmental health stressor due to the relatively elevated concentrations in several types of microenvironment, and in particular in occupational settings such as gasoline filling stations. The present study included an amount of field measurements, as well as model development and evaluation for the purpose of building a computational platform, which utilizes environmental data that can be obtained by automated remote sensors and performs risk assessment in real time for the critical working groups such as filling station employees.

In order to perform these calculations, the determination of exposure is a critical point. The ANN developed based on the field measurements constitutes a reliable modeling technique with high quality results for this purpose as indicated by the evaluation parameters in Table 2. Not all parameters measured in the field measurements campaign (benzene concentrations) where measured by automated sensors. Yet, benzene concentration measurements are needed only to train the ANN model – they are not needed in the platform for the latter to run. All the other parameters required as input for the model (ambient temperature, wind speed, amount of fuel traded, traffic flow, urban background concentrations) were or can be easily determined by sensors. In terms of expandability and applicability of the model, the exposure compartment could be applied to any filling station that the field measurement campaign is followed in an identical way, in order to obtain the new weights for the input parameters that may differ significantly from area to area, as shown in the results among the urban and the rural stations of the campaign.

On the contrary, the applicability of the PBPK model does not face any limitations, as it does not need any recalibration, since its optimization is based on experimental data related to given values of exposure to benzene, while the link to pathology and the related estimated risk are based on in vitro toxicological studies (Yokley et al., 2006). One other significant advantage of the PBPK model on that kind of applications is that it is a dynamic model, allowing fast and accurate response to changes of exposure concentrations, and consequently of the toxic metabolites at the target tissue (bone marrow) and finally to the estimated risk of leukemia.

From the environmental policy perspective, the general concept of the platform proposed herein is of great interest, since it can be the basis of different applications in similar environmental settings, where personal exposure to some contaminant is difficult to determine in real time but it is highly correlated to environmental parameters measured by automated real-time sensors. Furthermore, meta-analysis of real-time risk estimation can indicate how to change the time-activity patterns of humans in specific microenvironments where human exposure is the highest in order to reduce the estimated risk down to acceptable levels. A typical example is given by the distinction among the distributions of estimated disk among the employees performing different activities in the same venue. For instance, in a day when both ambient temperature and the amount of fuel traded are elevated and calm wind conditions prevail, exposure to benzene and estimated risk are significantly increased for employees performing car refueling. In this case, shift interchange among employees (in the form of rotation) could be recommended as a real-time measure to reduce cumulative health risk.

The proposed platform is a successful example of a scheme integrating different environmental sensors through data and model fusion algorithms in order to provide an effective tool for real time estimation of health risk to an occupationally exposed group. The algorithm structure requires a very small amount of environmental data (5 data classes used as input). This is due to the effectiveness of the individual models, which in turn is partially ascribed to the effectiveness of the Bayesian regularization algorithm in the development of models that describe linear and non-linear phenomena. Computational platforms like the one described in this study will be of increased interest in the forthcoming years due to the increasing public awareness about the interaction between environmental stressors and public health outcomes. We deem that precise health risk estimations given by similar approaches are proven to be more protective for occupational health than singly respecting ambient air limit values imposed by legislation.
References.

Figure captions.

Figure 1. Visual concept of the proposed risk assessment platform

Figure 2. Topography of the filling stations in combination to the prevailing wind

Figure 3. Structure of the proposed ANN.

Figure 4. Conceptual representation of the PBPK model.

Figure 5. Averaged weekly daily variation of traffic flow (in veh/h), fuel traded (in lt/h) and urban background concentration (in μg/m³) of the urban filling station.

Figure 6. Average weekly benzene exposure (in μg/m³) of the filling station employees for summer and winter respectively.

Figure 7. Averaged weekly daily variation of the exposure (in μg/m³) for the three categories of employees in summer and winter of urban (a) and rural (b) filling stations respectively.

Figure 8. Dispersion among the observed and the predicted values (in μg/m³) by the ANN for the exposure of the employees of urban (a) and rural (b) filling station respectively.

Figure 9. Relative importance of the parameters constituting the exposure pattern of the employees of urban (a) and rural (b) filling station respectively.

Figure 10. Estimated risk of leukaemia for the general population compared to the filling station employees.

Figure 11. Estimated risk of leukaemia for the general population compared to the filling station employees based on the individual main activities.
### Table 1. Percentage distribution of vehicle categories in each studied location.

<table>
<thead>
<tr>
<th></th>
<th>Urban</th>
<th>Rural</th>
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<tbody>
<tr>
<td>Catalytic</td>
<td>70</td>
<td>42</td>
</tr>
<tr>
<td>Non catalytic</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Diesel vehicles</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Light duty vehicles</td>
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<td>23</td>
</tr>
<tr>
<td>Heavy duty vehicles</td>
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<td>9</td>
</tr>
<tr>
<td>Buses</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Motorcycles</td>
<td>7</td>
<td>2</td>
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</table>

### Table 2. ANN exposure model evaluation parameters

<table>
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<tr>
<th>RRMSE</th>
<th>Employees refuelling cars</th>
<th>Employees of miscellaneous activities</th>
<th>Cashiers</th>
</tr>
</thead>
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<tr>
<td>Urban</td>
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<td>0.1081</td>
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<tr>
<td>Rural</td>
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<td>Urban + Rural</td>
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<td>0.0814</td>
<td>0.0556</td>
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<td>( R^2 )</td>
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<td></td>
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<tr>
<td>Urban</td>
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<td>Urban + Rural</td>
<td>0.9714</td>
<td>0.9919</td>
<td>0.9441</td>
</tr>
</tbody>
</table>
Figures.

**Figure 1**

**Urban Filling Station Topography**

**Rural Filling Station Topography**

**Washing Machines**

**Office**

**Fuel Pumps**

**Adjacent Road**

**Figure 2**

**Estimated Risk**
Figure 5

Figure 6