The low-dose benzene debate needs a sharp blade

Concawe's ongoing research into the health effects of benzene aims to address recently published findings on the low-dose effects of benzene and the potential impacts on the EU refining industry.

The health effects of benzene have been a major concern for regulators and health experts for many years. This has led to significantly lower regulatory threshold limits (such as occupational exposure limits, OELs[1]) and the implementation of corresponding risk management measures to reduce benzene concentrations and human exposure to benzene in the production, transport and use of petroleum products such as gasolines.

Over the past decade, a series of research papers has been published by a group of researchers at the University of California Berkeley who postulated effects of benzene at very low dose exposures (e.g. [1,2]). These low levels are relevant to current operations in the oil and gas industry and are currently (well) below the occupational exposure limits in most jurisdictions. However, the published findings on these low-dose effects have raised questions in the scientific community because the observations made are remarkable and report a relative increase in the intensity of the observed health effects at lower exposure concentrations; this is in contrast with the general rule in toxicology that 'the dose make the poison,[2] which implies that effects usually fade away as exposure concentrations get lower.

In these papers, the researchers pose that exposure to low concentrations of benzene (i.e. below 0.1 ppm) should be regarded as disproportionally hazardous. Because of these questionable findings and the potential impact on our industry, and since the scientific basis for the benzene OEL is presently under review in the EU, there is a need to verify the reported results in independent studies.

Concawe therefore has an ongoing research project which aims to shed new light on the reported benzene low-dose phenomenon. This project was initiated in 2016, starting with a re-analysis of the available evidence and the strength of the available data.

This first phase was completed in early 2017, with publication of the results in two peer-reviewed papers,[22,23] and indicates that the available data does not suggest an increased hazard from benzene at decreased exposure levels. These two papers have been shared with regulatory authorities to include in their (ongoing) assessments.

This Concawe review article on benzene is not intended to summarise the published papers from the Concawe project. It aims instead at providing the reader with a short overview of the scientific argumentation in the ongoing discussions on this topic as an example of an educational scientific debate; this is, incidentally, highly relevant in view of the ongoing OEL assessment for benzene in the EU, but also since the WHO's International Association for Research on Cancer (IARC) conducted a review on benzene in October 2017 in which it claimed that the low-dose effects of benzene are a major point of attention.

The following text is adapted from an article written by Prof. Dr Peter Boogaard (Shell, chair of Concawe's Toxicology Subgroup), which preceded the two Concawe publications mentioned above. The article, entitled 'The low-dose benzene debate needs a sharp blade', was published in a special section of the scientific journal *Chemico-Biological Interactions* (24 June 2017, e-publication ahead of print) and discusses the main aspects of the benzene low-dose debate in four major parts, addressing:

- metabolism of benzene;
- low-dose benzene measurement;
- low-dose benzene concentration calculation issues;
- the relevance of dermal exposure to benzene.

At the end of the article, the scientific debate is summarised and put into perspective.

No evidence exists that metabolism is different at low dose vs high dose benzene levels

Quantitative and qualitative differences in metabolism of certain compounds exist at low dose levels as compared to higher dose levels and this could potentially be due to the presence of a high-affinity, low-capacity enzyme. Indeed, the investigators reporting the low-dose phenomenon have postulated such an enzyme.[3,4] *However, this hypothetical enzyme has not been found yet.*[5,6] *Typically, such high-affinity, low-capacity enzymes play some crucial role in maintaining homeostasis or some other crucial vital physiological process and is phylogenetically well preserved across species. Nevertheless, to the best of my knowledge, this type of enzyme has never*

[1] An occupational exposure limit (OEL) is an upper threshold limit below which no human health hazards are to be expected, i.e. the maximum allowed concentration level of a potentially hazardous substance which is used to manage potentially dangerous exposures in the workplace.

[2] According to the first rule of toxicology, 'All things are poison and nothing is without poison; only the dose makes a thing not a poison'—an adage (translated from German) by Paracelsus, considered 'the father of toxicology'. *Paracelsus, dritte defension*, 1538.

been found for benzene (nor similar dose-dependent metabolism) in any animal species, therefore it doesn't seem very likely that humans would possess it.

Benzene exposure levels used to explain hypothesized effects were solely estimated, not measured

Another potential explanation could be found in the exposure assessment itself, that is if the claimed 'low dose' was actually not as low as it was deemed to be. The exposure data in the various publications go all back to a series of studies in China.[7,8,9] *If you have a closer look at the exposure assessments as reported in later studies (e.g.* [1,2,10]*), it is clear that in most of these publications actual exposure measurements were not done. On the contrary, the exposures are based on previously reported studies and essentially there is only one paper that forms the basis for the exposure assessment which is subsequently used in the other publications.*[11] *A closer look at this particular study shows that the low concentrations are not actually measured but rather calculated. According to the original paper where the methodology was described, the limit of detection of airborne benzene was 0.20 ppm.*[9] *All exposure values lower than this limit of detection of airborne benzene were calculated from the measured concentration urinary benzene using a correlation between airborne benzene and urinary benzene. The authors claim that the correlation they applied to do this was corroborated by the data of Ghittori* et al. *from 1993.*[12] *The paper by Ghittori and co-workers is a typical methodological paper in which they show that urinary benzene correlates reasonably well with airborne benzene concentrations when both values are log-transformed (r = 0.559 in 110 workers, both smokers and non-smokers; r = 0.763 in the 63 non-smoking workers only). Ghittori and co-workers, however, did not report a limit of detection. The lowest values measured were reported to be approximately 0.1 ppm, but the scatter, especially at lower concentrations, is rather large. In any case, the 'low-dose' concentrations are not actually measured directly as clearly stated in the Thomas* et al. *paper:*[1] *"For each of the exposed individuals in the study, benzene exposure was estimated in terms of the average air-benzene level (in units of parts-per million). The exposure levels of the 42 subjects that were below the limit of detection were estimated using un-metabolized urinary benzene levels, as previously described."*[11] *The McHale* et al. *paper*[2] *apparently uses the study population of the Lan* et al. *study*[8] *for which the exposure assessments were done according to Vermeulen* et al.[9,13]

Low dose benzene levels that were used to proof non-linearity were calculated using linear statistical models

If one has a look at the figures in the publication by Kim and co-workers where the dose related production of urinary metabolites is given as a function of the median value for airborne benzene concentration (Figure 4 in [11]*), it is obvious that for most metabolites only the data points between 0.01 and 0.1 ppm benzene are not 'in line'. The most obvious reason seems to be that the airborne benzene concentrations related to these data points are calculated and not measured unlike the airborne benzene levels for the other data points, as explained above. The data are based on measured urinary benzene levels using a simple linear regression model: basically airborne benzene concentrations are linked to urinary benzene levels. In general, that is a valid approach, but, in my view, it is fundamentally wrong to use this linear equation subsequently to demonstrate non-linearity in metabolism for low exposure levels. If you assume that metabolism is different (i.e. essentially non-linear) at concentrations less than 1 ppm, you cannot use a linear regression between airborne benzene levels greater than 1 ppm and urinary benzene (or any urinary metabolite) to predict airborne benzene less than 1 ppm as the amount of un-metabolised benzene in urine is no longer independent under your assumption.*

Dermal exposure, which is probably the most realistic exposure route given the occupational setting under evaluation, is completely dismissed

Another question rose with regard to potential other routes of exposure, especially skin exposure. The Vermeulen et al. *paper*[9] *explicitly states that dermal exposure is not expected to have contributed to the total exposure: "Preliminary analyses of dermal exposure data collected as part of the current study indicate that this route of exposure did not contribute substantially to the*

total benzene and toluene doses received (unpublished data)". Actually, these data were published a couple of years later.[13] *In this paper the authors describe the dermal monitoring of 70 individuals involved in 6 different tasks using dermal patches. However it is not reported how many persons that were monitored were involved in each of the tasks. In a number of individuals (3 for benzene and 5 for toluene), one or more of the patches indicated that dermal exposure might have occurred and, without exception, these persons were involved in the same task 'gluing'. The authors admit that dermal exposure might have been missed since only a very limited area of the skin was covered by the patches and the spatial distribution of dermal exposure was expected to be non-uniform. Nevertheless, because a strong association between airborne benzene and benzene in urine was found, it was concluded that inhalation was the predominant route of exposure. The authors then support the plausibility of their conclusion by quoting US EPA documentation on benzene that dermal absorption of benzene is usually negligible. However, the assumption by US EPA that dermal absorption of benzene is between 0.05 and 0.1% is dubious, if only since it is not specified what this percentage refers to: neat benzene on the skin, benzene vapour through the skin, dermal absorption as percentage of the inhaled amount. All of these aspects are important and it seems that this assumption is actually based on the IRIS documentation on dermal absorption of benzene, which is simply incorrect as I've argued before.*[14] *In fact, most regulatory authorities have assigned a skin notation to benzene, which implies that in occupational settings dermal uptake is more than 10% of the uptake by inhalation.*[15] *Assuming that it is less than 0.1% seems untenable. There are a few recent reviews on the dermal uptake of benzene*[16,17] *and there seems to be consensus that the dermal flux for benzene is between 0.2 and 0.4 mg/(cm^2.h). Hence, if a flux of 0.3 mg/(cm^2.h) is assumed—which is low, since the benzene is in glue, see below—and make the same assumption as was done in the paper that 10% of the surface of both hands (36 cm^2) was contaminated, the estimated uptake would be 10.8 mg/h, or 86.4 mg of benzene over an 8-h working day, which is quite a bit higher than the ~ 0.5 mg that was suggested in the paper.*[13] *In addition, it should be realized that most assumptions for dermal uptake of benzene apply to neat benzene which is expected to be different from benzene in glue. Available data indicate that aqueous benzene solutions behave similar to neat benzene, probably since the benzene is volatile and lipophilic. However, benzene dissolved in organic solvents (hexane, gasoline, and probably glue) has a more variable flux, but generally the organic matrix enhances skin penetration, which may be expected as the benzene won't evaporate as easily.*[16] *Hence, dermal uptake of benzene seems quite feasible to have occurred to some extent, especially during 'gluing'. This might explain one of the conclusions from the re-analysis of the data by McNally* et al.[22] *that "some aspect of exposure was not captured by a full shift air sample".*

In summary: the low dose benzene debate, and why it would benefit from a sharp blade of Ockham's razor [3]

Even if we ignore the arguments about the mysterious high-affinity, low-capacity enzyme as well as the potential dermal exposure that may have played a role, and we also disregard the fact that the lowest airborne concentrations are not actually measured, but just take the actual exposure data, as reported in the papers by Kim and co-workers,[8,9] *at face value, the low-dose phenomenon is still not immediately obvious. Therefore, the original data from these studies as well as their modelling as performed by Kim* et al.[7] *were reanalyzed by Price* et al.[18] *Price and co-workers addressed several critical technical issues, such as the corrections applied for metabolite background levels and the calibration model applied to estimate airborne benzene concentrations for certain workers, and concluded that there was no statistically significant departure from linear metabolism at low exposure concentrations. Rappaport and co-workers reacted furiously to this critique*[19] *and Price and co-workers, in turn, reacted to the response by Rappaport* et al.[20] *providing additional analysis as to why both the original claim of low-dose specific metabolism and the rebuttal comments offered by Rappaport and co-workers remained highly implausible and speculative. One area of great attention arising from these public debates is the risk of conflict of interests that may occur for all stakeholders involved in these applied research programmes since these novel claims of increased risk of attracting leukemia by exposure to benzene at much lower levels than previously assumed to pose a carcino-*

[3] Ockham's razor is a principle attributed to the 14th century philosopher William of Ockham, which states that, 'Entities should not be multiplied unnecessarily'—or in other words, when you have two competing theories that make exactly the same predictions, the simpler one making fewest assumptions is the better.

genic risk will most probably lead not only to increased benzene health-related litigation, but also to calls for regulatory action to further lower acceptable benzene exposures. Therefore, both the scientists conducting research and studies on behalf of industry and academic researchers, whose funding is generally provided by regulatory bodies and governmental institutes (US EPA, OSHA, NIEHS, NCI, NIOSH) and who act as expert-witness in benzene-litigation cases,[6, 11, 13, 19, 21] *are likely to be subject to the risk of conflict of interest. As a result and in order to avoid any risk of conflict of interests, great care should be given by all involved stakeholders to develop conclusions that are built on correct and well supported scientific arguments.*

It was therefore considered important that the data would be independently reanalyzed by two different research groups: Cox Associates and the UK Health & Safety Laboratory. The two research groups followed a very different approach in re-analysing the data but both came to the conclusion that, although the data reported in the studies that led to the hypothesis of the low-dose benzene phenomenon indeed do not exclude non-linear metabolism at lower concentration of benzene, the data are also fully consistent with the absence of any non-linearity in benzene metabolism at low doses. Since the absence of non-linearity does not require hypothetical enzymes or any other unproven assumption, it would be the preferable scientific stance according to Ockham's razor.

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