

RIVM-advies

On the reliability and validity of the INTRON report by Industox 2006 as questioned by Prof. Dr. Berger.

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Introductory note:

The RIVM was requested by of the Ministry of Housing, Spatial planning and the Environment (presently: Ministry of Infrastructure and the Environment) in 2006 to participate in the steering committee of the INTRON study. The aim was to focus on the review of the test design and test results in 2006, without having any influence in the execution of the study by IndusTox.

Briefly, the INTRON study performed by IndusTox concerned the analysis of polycyclic aromatic hydrocarbon (PAH) exposure in urine of soccer players on the day before, during and after intensive contact with rubber granules on artificial turf. The study was performed with seven volunteers given instructions on dietary restrictions and an intensive exercise on the day of exposure. Further, the volunteers were asked to collect their urine on these three days. A biomarker, 1-hydroxypyrene in urine, was used to estimate the internal PAH exposure. According to the study authors this biomarker is sensitive to detect even low PAH exposure. Based on the study results, the authors concluded that intensive contact with the rubber granules did not result in a clear and uniform dermal uptake of PAHs in the volunteers. Even if there was a slight uptake of PAHs from the rubber granules, it was concluded to be limited and within the range of background exposure.

In a letter dated February 9th of 2007, the RIVM informed the Ministry of Housing, Spatial planning and the Environment of her findings regarding the study. A copy of this letter is attached for your information, and can be summarized as follows: in 2007, RIVM findings were that the INTRON study provided valuable results for the human health and environmental risk assessment of dangerous substances in rubber granules used as infill material in artificial turf (RIVM, 2007). RIVM noted that the results were in line with previous conclusions by RIVM (RIVM, 2006), stating that PAHs are released from rubber granules, however based on the available information this does not result in a foreseeable health risk. With regards to the INTRON study, one important remark was made, namely on the exclusion of biomonitoring data by INTRON because they were outside the range of normal creatinine levels. This was considered questionable as athletes may have increased creatinine levels after exercise. Although all data were provided, calculations with the data outside the range should also have been presented (individual data are presented in Appendix 9 of the INTRON report (September 2006)). Finally, it was anticipated that even with the out of range data, if corrected for increased creatinine levels (based on athlete creatinine data), the same conclusion on human health risks from PAHs in rubber granules can be drawn (RIVM, 2007).

Our involvement in the review of the test design and results may be interpreted as a conflict of interest with regard to the present questions. However in the following you will find our review of the comments of Prof. Dr. Berger and our updated opinion on the INTRON report.

With regard to documents:

The INTRON report prepared by Industox (23 dec 2006).

Comments by Prof. Dr. M.P.F. Berger on the INTRON report.

RIVM letter dated February 9th of 2007, addressed to mr. A.B. Holtkamp at the Ministry of Housing, Spatial planning and the Environment.

Questions

1. Assess the comments made by Prof. Dr. Berger on the INTRON report questioning the reliability and validity of the conclusions in the INTRON report:
 - a. Representativeness of sample size
 - b. Skin contact in exposure protocol is not uniform.

- c. Questionable reliability of the results because of increased marker values prior to the exposure and decrease of levels after exposure.
 - d. Outdoor temperature is too low.
 - e. There is an incomplete processing of the data, because data outside of range of normal creatinine levels have been left out for further consideration.
 - f. There is a lack of information on the control group used in the study.
2. What is the RIVM opinion on the INTRON report by Industox (September 2006)

RIVM response

Ad 1. Assess the comments made by Prof. Dr. Berger on the INTRON report questioning the reliability and validity of the conclusions in the INTRON report:

1a) Representativeness of sample size.

Comment by Prof. dr. Berger: The sample size of seven volunteers is too low to cover the large number of athletes playing on artificial turf on a weekly basis. In addition, the volunteers are adult males only. Moreover, Prof. dr. Berger states that even if the sample size should be considered representative, it would imply that 1 out of 7 volunteers (or maybe even 1 out of 4 volunteers due to the use of massage oil (see point 1b)) does show elevated PAH levels in urine, which would mean that 14% of the athletes on artificial turf with rubber granules have elevated PAH levels after contact with the material.

Description in INTRON study: The number of volunteers included in the INTRON study was seven soccer players, of which all males, non-smoking, aged 20 to 31 years, weighing 67 to 104 kg. The control group used in this study originates from 1992, including only males, of which 39 non-smokers and 37 smokers. This information provides confidence about the normal range of background levels of the 10 PAHs included in the study.

Remarks by RIVM: The number of athletes included in the study is indeed low and cannot be considered to be representative for all athletes, also including children and women. As a consequence the statistical power of the study is too low to conclude on the overall safety of athletes playing on artificial turf with rubber granules (rubber infill material). Please also refer to RIVM's remarks under point 1b.

Ad 1b) Skin contact in exposure protocol is not uniform.

Comment by Prof. dr. Berger: Prof. Dr. Berger remarks that the protocol for skin contact is not uniform amongst the volunteers. Firstly, the intensity of the skin contact is dependent on the volunteer and secondly, three of seven volunteers were treated with massage oil (Chemodol ®), which according to the INTRON study authors may affect the PAH uptake in a positive way. Prof. Dr. Berger further notes that the INTRON report does not specify which volunteers were treated with the massage oil.

Description in INTRON study: The volunteers in the study were given the assignment to perform exercises of in total 30 minutes prior to warming-up and the match. They have been given instructions to do 2 slidings per leg, to sit for 10 minutes with stretched legs and to crawl for another ten minutes. After pre-structured exercise, a warming-up and soccer match followed for which no further instructions were given. Three of seven players were treated with massage oil (Chemodol ®), which is often used and is hypothesized to enhance dermal uptake of PAHs. The report states it concerned subjects A, B and D (see page 6, Table 1 of the report).

Remarks by RIVM: With respect to the intensity of the dermal contact, RIVM disagrees with Prof. Dr. Berger that the contact is non-uniform. The intensity of contact is indeed determined by the player, which cannot be contested, but this does not address non-uniformity, but rather intra-volunteer differences.

With respect to the use of massage oil: in fact, these three volunteers should be regarded as a separate group, unless results show that the group is similar to the main group. This was not studied by the authors of the INTRON study.

Unlike Prof. Dr. Berger states, the subjects treated with massage oil are indicated in the report (A, B and D, see page 6, Table 1 of the report). Interestingly to note is that for subjects B (statistically significant) and D an increase of the biomarker, compared to their own background levels, was observed after contact with the rubber infill material. It should be noted that individual D showed a relatively high background level, probably caused by occupational and/or dietary sources. The authors did not consider the possibility of the massage oil as cause for the increase of the biomarker, but speculated that eating a hamburger later on the day may have caused the increase.

This was previously shown by Van Maanen et al. (1994) that eating of grilled meat can increase 1-hydroxypyrene levels.

The inclusion of the massage oil should have led to separate analyses of the sub-group to evaluate the possible influence on PAH uptake on the one hand, and to proof that the results from all seven subjects can be considered as one group or not. If not, it would mean that the group sizes and consequently the statistical power was even lower. RIVM agrees that the exposure resulting from dermal contact with and without massage oil cannot be considered as uniform.

Ad 1c) Questionable reliability of the results because of increased biomarker values prior to the exposure and decrease of levels after exposure.

Comment by Prof. dr. Berger: The data of three volunteers are considered to be of questionable reliability as the biomarker levels prior to the sporting event were relatively high and significantly decreased after the sporting event. Since these findings are contradicting the expectations and no conclusive explanations were provided, the data should have been marked unreliable and left out for further analyses and evaluations.

Description in INTRON study: The INTRON study stated that three subjects (D, E and F) show relatively high background levels (statistically significant higher than 95th percentile of the control group) prior to contact with the rubber infill material. Although no clear explanation could be given, it was considered possible that occupational and/or dietary sources caused the increased background levels based on activity statements by the volunteers. The urine samples after contact with the rubber infill material showed a decrease in marker levels, compared to their individual background levels (and below the 95th percentile of the control group, but higher than the geometric mean). The subjects were considered in further analyses.

Remarks by RIVM: RIVM agrees with prof. Dr. Berger in the correctly stating that the background levels of the three subjects should not be considered reliable in view of the comparison between individual background levels vs. post-exposure levels. However, RIVM disagrees to discard the data for these subjects altogether. If one looks at the post-exposure levels only of these subjects, they are relatively high (at similar level as for subject B for which it was concluded that biomarker levels were statistically significant elevated after dermal contact), but remain below the 95th percentile of the control group. Noteworthy, if the authors included the data outside the range for subject F there would not be a decrease at all (see point 1e on incomplete processing of all available data). In cases of subjects D and E, there would still be a decrease. Secondly, it should be noted that the biomarker levels of subject D, E and F, show an upward trend after the sporting event, before declining again. In the view of RIVM, the authors should have marked the individual background data of subjects D, E and F as unreliable, and use the control group data as surrogate background levels to make the comparison between background and post-exposure biomarker levels (and to do this for all subjects). It is to be noted that it would mean that not one, but four subjects showed an increase in biomarker levels after exposure, but would still lie in the range of normal background levels of the biomarker levels of non-smokers.

Ad 1d) Outdoor temperature is too low

Comment by Prof. Dr. Berger: Prof. Dr. Berger expresses concerns that the outdoor temperature during the study was too low, where higher exposures to PAHs are anticipated during summer activities.

Description in INTRON study: The temperature during the study was 17 to 18 °C (in September 2006).

Remarks by RIVM: Indeed, under elevated outdoor temperatures it may be anticipated that the release of PAH from rubber infill materials is enhanced. The PAHs are not chemically bound to the rubber and as a result may be released easier under elevated temperatures. The release or desorption of PAH from soil is significantly decreased under lower temperatures (Enell et al. 2005) giving support to the concern. The study would have benefitted from taking urine samples on various days, including hot summer days, as it would make the dataset more representative for all possible exposure situations. It is noted though, that the outdoor temperature may not be the worst case situation, it does resemble a higher than average outdoor temperature in the sporting season (it is just under the average maximum temperature in the Netherlands of 22 °C (in July and August) (www.klimaatinfo.nl/nederland, accessed 20-Jan-2016).

Ad 1e) There is an incomplete processing of the data, because data outside of range of normal creatinine levels have been left out for further consideration.

Comment by Prof. Dr. Berger: Prof. Dr. Berger remarks that there is incomplete processing of the data, because data outside of range of normal creatinine levels have been left out for consideration and calculation. The INTRON report does not specify which data are concerned.

Description in INTRON study: From the 7 subjects and 138 measurements or data points, all data with a creatinine level (used for correcting for the amount of urine) outside of range of normal

creatinine levels were excluded. This was valid for a total of 25 data points, and at least one sample outside range in all subjects.

Remarks by RIVM: As noted previously in 2007 by RIVM, this approach was considered questionable as athletes may have increased creatinine levels after exercise. Although all data were provided, unlike Prof. dr. Berger stated, also calculations with the data outside the range should have been presented (for the individual data they are presented in Appendix 9 of the INTRON report). Finally, it was anticipated that even with the out of range data corrected for increased creatinine levels (based on athlete creatinine data) the same conclusion on human health risks from PAHs in rubber granules can be drawn.

Ad 1f) There is a lack of information on the control group used in the study.

Comment by Prof. dr. Berger: it is stated that there was a lack of information on the control group.

Description in INTRON study: The control group used in this study stems from 1992, including only males, of which 39 non-smokers and 37 smokers and are all from the Nijmegen region in The Netherlands (where the study was performed, Malden). The control group is not occupationally exposed to PAHs. Further details have not been provided.

Remarks by RIVM: The control group can be considered as an historical control group rather than the traditional control group generally seen in e.g. animal studies, where exact handlings have been performed without the test substance. Even though exact details are not provided about how the control group was potentially exposed to sources of PAHs, which is indeed an omission, one may find use in the information that is provided that the control group is not occupationally exposed and thus only exposed via dietary and residential sources. Further, the control group is used only to put the results in perspective (especially the individual background marker levels), rather than making comparative analyses with the control groups. It is not considered a major drawback of the study. As remarked under 1c, RIVM states that a comparison with the control group would have been a valuable addition to the report. In that case, more information on the control group should be provided for proper analyses and drawing of conclusions.

Ad 2) Opinion of RIVM on the INTRON study

In 2007, RIVM findings were that the INTRON study provided valuable results for the human health and environmental risk assessment of dangerous substances in rubber granules used as infill material in artificial turf (RIVM, 2007). RIVM noted that the results were in line with previous conclusions by RIVM (RIVM, 2006), stating that polycyclic aromatic hydrocarbons (PAHs) are released from rubber granules, however based on the available information, this does not result in a foreseeable health risk. With regards to the INTRON study, one important remark was made, i.e. on the exclusion of biomonitoring data because they were outside the range of normal creatinine levels. This was considered questionable as athletes may have increased creatinine levels after exercise. Although all data were provided, calculations with the data outside the range should also have been presented (individual data are presented in Appendix 9 of the INTRON report (September 2006)). Finally, it was anticipated that even with the out of range data, if corrected for increased creatinine levels (based on athlete creatinine data), the same conclusion on human health risks from PAHs in rubber granules can be drawn (RIVM, 2007).

RIVM is of the opinion that the INTRON study provides valuable data for human health risk assessment of PAHs in rubber infill material on artificial turf. In our view the study was well performed technically, but does have limitations regarding its representativeness to athletes playing on artificial turfs.

Additional remarks:

- i) Children and women were not studied in the INTRON study, and the study report did not include a justification to limit the study population to men.
- ii) Other artificial turfs and rubber infill materials used on soccer fields have not been included in the study and therefore, the conclusions of the report are limited to the artificial turf and rubber infill material used in the INTRON study. The report makes no statement regarding the representativeness of the rubber infill material for other types of infill material.
- iii) The lowest observed effect level (LOEL) used in the INTRON study as health based limit value is based on a Belgian research including workers occupationally exposed to PAHs. It is uncertain if the LOEL is applicable to the general population as well, where generally lower health limit values are used to cover for intra-individual differences. As was previous concluded in the PAH restriction dossier: "children are more vulnerable

- than adults". The LOEL in the INTRON report is a biological limit value, for which no counterpart could be found in the PAH restriction dossier. Therefore, it was not possible to extrapolate the DMELs for the general population to a similar biological limit value.
- iv) The assigned exercises prior to warming-up and the match indicate a rather worst case contact scenario with the rubber infill material as was noted by the volunteers never been that 'dirty' before from the material. It can be concluded that the exposure scenario was worst case, although it could be worse under elevated temperatures. Moreover, the exposure does not occur on a daily basis and is typically less than the 2.5 hours contact in the INTRON study.
 - v) Regardless of the identity of the source responsible for the elevated background marker levels in subjects D, E and F, it illustrates that others sources have far more effect on PAH uptake than the rather worst case exposure to the rubber infill materials.
 - vi) Since 2007 several new scientific studies have been carried out, especially in the United States. The information from these studies has not been taken into account.

Based on the evaluated information we conclude that polycyclic aromatic hydrocarbons (PAHs) are released from rubber granules that can lead to increased internal PAH levels. However, this does not indicate a foreseeable health risk for athletes sporting on artificial turf.

Final notes and recommendation

The ECHA guidance on the interpretation of the PAH restriction (entry 50 under REACH) is still under discussion. It was discussed at CARACAL in 2015, but this did not result in a final guidance document. The interpretation obviously affects whether or not rubber infill material is covered by the restriction.

The PAH restriction dossier (BAuA, 2010) submitted to the European Commission to urge the Commission to apply REACH article 68.2 contains examples for rubber granules at playgrounds, indicating that under intensive contact they may not be safe for children. Daily contact was assumed where children would be exposed to the rubber granules when coming from a slide, 50 times with short contact of 30 seconds. Exposure was modelled using varying input options for migration rates (release data was unknown). However, it was noted that the calculations are very conservative and uncertain, expressing the need for more PAH release data (studies included up to 2008). BAuA did not take this scenario forward in the risk assessment, because of the large uncertainties.

We recommend the evaluation of new information published since the publication of the INTRON study in 2006, taking into account the information as included in the PAH restriction dossier (BAuA, 2010).

References

BAuA (2010). Annex XV restriction report. Proposal for a restriction benzo[A]pyrene, benzo[E]pyrene, benzo[A]anthracene, dibenzo[A,H]anthracene, benzo[B]fluoranthene, benzo[J]fluoranthene, benzo[K]fluoranthene, chrysene. Version 1, date 31052010.

Commentaar door Prof. dr. Berger op: IndusTox (2006). Urineonderzoek naar PAK-blootstelling bij voetballers op kunstgrasveld ingestrooid met rubberkorrels. INTRON study (in Dutch).

Enell A, F Reichenberg, G Ewald, P Warfvinge (2005). Desorption kinetics studies on PAH-contaminated soil under varying temperatures. Chemosphere. Volume 61, Issue 10, December 2005, Pages 1529-1538.

IndusTox (2006). Urineonderzoek naar PAK-blootstelling bij voetballers op kunstgrasveld ingestrooid met rubberkorrels. INTRON study. Vertrouwelijk (in Dutch).

RIVM (2006). Brief aan Ministerie van VROM, m.b.t. rubbergranulaat dossier. SEC-060950.

RIVM (2007). Brief aan Ministerie van VROM, m.b.t. rubbergranulaat dossier. SEC-070105.

Van Maanen et al. (1994). Formation of aromatic DNA adducts in white blood cells in relation to urinary excretion of 1-hydroxypyrene during consumption of grilled meat. *Carcinogenesis*. 15:2263-2268.