Mode of Action (MoA) of stone-induced urinary bladder tumours with species-specific aspects and its relevance for humans

GENERAL VCI-COMMENT TO THE ROPOSAL FOR A HARMONISED CLASSIFICATION AND LABELLING OF MELAMINE (1,3,5-TRIAZINE-2,4,6-TRIAMINE)

Germany submitted a "harmonised classification and labelling" (CLH) dossier to propose a future entry for Melamine in Annex VI of CLP Regulation as "Carc. 2 (H351) and STOT RE 1 (H372). In this paper the VCI will give a general comment to the Mode of Action (MoA) of stone-induced urinary bladder tumours with species-specific aspects and its relevance for humans.

Background to urinary tract solid formation

Urinary tract solid formation is a threshold phenomenon, with a physico-chemically defined dose required to attain crystallization in the urinary tract. If the dose is insufficient to lead to crystallization, there is no cytotoxicity, no regenerative proliferation, and finally, no tumor formation.

Tumor formation requires long-term presence of the urinary solids. The concept of crystals and calculi leading to the toxicity and carcinogenicity of several chemicals was first discussed in 1974, based on experiments performed at that time on a sulfonamide drug implanted into the bladders of mice.[1]

Urinary tract crystals and calculi can occur in rodents, pets, and humans by a variety of substances, including the essential ingredients in our diet (calcium, magnesium), a variety of drugs (acetazolamide, sulfonamides, and HIV protease inhibitors), and a variety of chemicals. Many of these substances have produced urinary tract tumors in rodents, especially in rats only, so that the risk assessment is entirely on the

[1] Clayson, D. B. Bladder carcinogenesis in rats and mice: Possibility of artifacts. Journal of National Cancer Institute, 1974, 52: 1685-1689.

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relationship of urinary solids to tumors, rather than on the intrinsic properties of specific chemicals.[2,3]

This fact has been reviewed over decades by a wide variety of agencies, including the International Agency for Research on Cancer (IARC), the US Environmental Protection Agency, the European Chemical Agency (ECHA), European Medicines Agency, Food and Drug Administration, and Health Canada, amongst others. Overall, the general conclusion of expert-panel evaluations has been that crystals and calculi pose a threshold toxicity hazard in particular in rodents, but not a real carcinogenic risk for humans.

Carcinogenic effect of urinary tract solids in male rat and humans

Calcium-containing salts are the most frequent calculi that form in the human urinary tract. Obviously, calcium is not considered carcinogenic but at sufficiently high doses the extensive regulatory network responsible for calcium homeostasis can lead to formation of calculi in humans. The basis for the difference in susceptibility to carcinogenesis from urinary solids has not been completely explained, but nevertheless, is a consistent finding in animal testing.

During animal testing, rodents – and especially the male rat, appears to be the most susceptible species, followed by the mouse.

There are several reasons why the urinary tract of humans is not susceptible to the carcinogenic effect of urinary tract solids. It ultimately comes down to a variety of anatomic differences between rodents and humans.[4] In rodents, calculi can accumulate in the dome of the bladder without complete obstruction of the urinary tract, so that the calculi can remain present in the urinary tract essentially for the lifetime of the animal without complete kidney deterioration.

In contrast, in the human urinary tract, calculi produce complete obstruction of the urinary tract. This is partly due to the vertical nature of human stature so that stones cannot accumulate in the bladder, but also because of specific anatomic factors in humans. Specifically, there are several points along the urinary flow that have

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[2] Known substances producing urinary calculi when administered to rodents and/or humans include e.g. Uracil, Thymine, Fosetyl-al, Melamine, Sulfonamides, Ampicillin, Amoxicillin, Urate, Homocysteine, Cysteine, Calcium Oxalate, Calcium Phosphate, Calcium Carbonate, Diethylene Glycol, Oxamide, Acetazolamide, Terephtalic acid, Dimethylterephthalate, Polyoxyethylene-8-stearate, Glycine, Pyroxasulfone, Sulfosulfuron, Muraglitazar, Pioglitazone, 4-Ethylsulfonylnaphthalene-1-sulfonamide, Salicylazosulfapyridine, Disperse blue 1, Triamterene, Indinavir, Glafenic acid, Orotic acid.

[3] Cohen, S. M. Screening for human urinary bladder carcinogens: two-year bioassay is unnecessary. Toxicology Research, 2018, 7(4): 565-575.

[4] DeSesso, J. M. Anatomical relationships of urinary bladders compared: Their potential role in the development of bladder tumors in humans and rats. Food and Chemical Toxicology, 1995, 33: 705-714.

significant narrowing of the urine passage so that stones can cause obstruction at these sites.

These include the site at which the kidney pelvis narrows to become the ureters, secondly, at the point where the ureters cross the pelvic brim, and thirdly, the traversing of the ureter through the urinary bladder muscular wall.

If urinary crystals and small calculi, from whatever origin, form, they will readily be excreted without obstruction, without damage to the urothelium, and will not accumulate in the urinary bladder. Crystalluria in humans does not have the same effect it can have in rodents where cytotoxicity of the urothelium can occur with consequent regenerative proliferation.[5] In contrast, in humans, crystals either have no effect, or they are a predictor of the formation of calculi. By themselves, the crystals do not appear to produce toxicity of the human urothelium. If they accumulate in the renal tubules, then toxicity can occur, which has been most widely studied in the relationship of calcium oxalate crystals forming secondary to the ingestion of large quantities of ethylene glycol (antifreeze).[6]

Thus, substances such as sodium saccharin, sodium ascorbate (vitamin C), sodium bicarbonate (baking soda), sodium chloride (table salt), and a variety of other sodium salts can produce crystalluria in rats leading to the formation of bladder tumors, but this does not occur in humans.[7,8]

Likewise, a variety of crystals that occur in humans by themselves are not toxic to the urinary tract, such as uric acid, calcium phosphate, calcium oxalate, calcium carbonate, a variety of magnesium salts, and others, unless they form calculi.

If calculi actually do form in humans and are large enough to obstruct the urinary tract rather than being immediately excreted in the urine, complete obstruction occurs resulting in excruciating pain. When this happens, the calculi are either spontaneously voided following ingestion of large amounts of liquids, or they are removed ultrasonically or by surgery. This is a major difference between the effects in rodents and humans since the urinary tract tumor formation is dependent on the long-term presence of these urinary crystals and calculi in the rodents.

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[5] Dominick, M. A. *et al.* Urothelial carcinogenesis in the urinary bladder of male rats treated with muraglitazar, a PPAR alpha/gamma agonist: Evidence for urolithiasis as the inciting event in the mode of action. Toxicol. Pathol., 2006, 34(7): 903-920.

[6] Seo, J.W. *et al.* Acute oxalate nephropathy caused by ethylene glycol poisoning. Kidney Res. Clin. Pract., 2012, 31:249-252.

[7] IARC. Consensus Report. International Agency for Research on Cancer. IARC Scientific Publications, 1999, 147: 1-32;

[8] Cohen, S. M. Screening for human urinary bladder carcinogens: two-year bioassay is unnecessary. Toxicology Research, 2018, 7(4): 565-575.

Regulatory considerations

Overall, the weight of the evidence approach leads to the conclusion that calculi by themselves are not carcinogenic to the human urinary tract. ECHA has acknowledged this fact by including in its Guidance mechanisms of tumor formation that are not relevant for humans[9]:

> *3.6.2.3.2. Additional considerations for classification, letter (k):*
>
> *Mode of action and its relevance for humans, such as mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression*
>
> *Some mechanisms of tumour formation considered not relevant for humans:*
>
> - Kidney tumours in male rats associated with substances causing α2μ-globulin nephropathy (IARC, 1999)
> - Pheochromocytomas in male rats exposed to particulates through inhalation secondary to hypoxemia (Ozaki et al, 2002)
> - Leydig cell adenomas induced by dopamine antagonists or gonadotropin-releasing hormone (GnRH) (EU Specialised Experts, 2004; RIVM, 2004)
> - ***Urinary bladder tumours due to crystals in the bladder (IARC, 1999)***
> - Forestomach tumours in rodents following administration by gavage of irritating or corrosive, non-genotoxic substances (RIVM, 2003; IARC 2003)
> - Certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (IARC, 1999; EU Specialised Experts, 1999)
> - Liver tumours in rodents conclusively linked to peroxisome proliferation (IARC, 1994)

Examples for mechanisms considered not relevant for humans by ECHA include e.g. urinary bladder tumours due to crystals in the bladder. All mentioned exemptions, including references to kidney and liver tumors, refer to findings in rodents and in some cases more specifically to rats (predominantly male rats). Therefore, some mechanisms, including bladder tumor formation, can be considered a species-specific phenomenon and must be excluded from classification for carcinogenicity due to their non-relevance for humans.

<u>Conclusion:</u>

- Many chemicals are known to form crystals in the bladder, yet are not considered to be intrinsically carcinogenic. Instead, threshold driven risk assessment is appropriate to address the concern of stone builders.
- Calculi dependent bladder tumor formation is a secondary effect dependent only on the physicochemical solubility of a substance that cannot be attributed to an intrinsic toxicity of a substance but requires long-term bladder stone exposure.

[9] Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 5.0, July 2017

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- Furthermore, the tumor formation after stone-building seems to be species- and gender-specific (primary male rate) due to anatomical difference between rodents and humans.

Contact: Dr. Denis Pahlke, Referent, Dept. Science, Technical and Environmental Affairs
Phone: +49 (69) 2556-1637
E-Mail: pahlke@vci.de
Internet: www.vci.de · *Twitter:* http://twitter.com/chemieverband · *Facebook:* http://facebook.com/chemieverbandVCI

German Chemical Industry Association
Mainzer Landstrasse 55, 60329 Frankfurt, Germany

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