AAPS PharmSciTech (2019) 20: 166
DOI: 10.1208/s12249-019-1376-1

Review Article

Theme: Team Science and Education for Pharmaceuticals: the NIPTE Model
Guest Editors: Ajaz S. Hussain, Kenneth Morris, and Vadim J. Gurvich

Lesson Learnt from Recall of Valsartan and Other Angiotensin II Receptor Blocker Drugs Containing NDMA and NDEA Impurities

Naseem Ahmad Charoo,[1] Areeg Anwer Ali,[2] Shailesh Kumar Buha,[3] and Ziyaur Rahman[4,5]

Received 4 February 2019; accepted 25 March 2019; published online 15 April 2019

Abstract. The presence of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) impurities in angiotensin II receptor blocker (ARB) drugs containing tetrazole ring has triggered worldwide product recalls. The purpose of this article is to identify the potential gap area in current pharmaceutical industry practice that might have led to the NMDA and NDEA impurities escaping the drug manufacturer's and FDA's attention. The impact of process change was not adequately assessed by the manufacturer of contaminated APIs (active pharmaceutical ingredients), and potential for generation of mutagenic or other toxic impurities was not considered. The safety and risk associated with a chemical synthetic process was also not evaluated. This is primarily due to current industry practice which focuses on controlling the impurities above reporting threshold. ICH Q3A and FDA guidance on genotoxic and carcinogenic impurities in drug substances and products need to be integrated so that the ICH Q3A decision tree (attachment 3) begins by checking whether the synthetic process has been evaluated for the potential to generate toxic impurities. The compliance with ICH Q3A limits should be carried out only after the process has been determined to be safe without the risk of generating mutagenic and carcinogenic impurities.

KEY WORDS: N-nitrosodimethylamine; N-nitrosodiethylamine; valsartan; losartan; irbesartan; product recall; carcinogenic; FDA; ICH.

INTRODUCTION

The presence of N-nitrosodimethylamine (NDMA) in valsartan tablets triggered a voluntary product recall on 13th July 2018 for valsartan finished formulations manufactured by Teva Pharmaceutical Industries, Major Pharmaceuticals and Solco Healthcare. The common thread among these companies recalling their products was valsartan API (active pharmaceutical ingredient) manufactured by Zhejiang Huahai Pharmaceuticals (ZHP), Linhai, China. Subsequently, another carcinogenic impurity N-nitrosodiethylamine (NDEA) was found in another valsartan product manufactured by Torrent Pharmaceuticals who had voluntarily recalled the product on August 23, 2018. The Torrent product also contained valsartan API manufactured by ZHP (1). ZHP was reported to have found the NDEA impurity in several valsartan API batches prompting the FDA (Food and Drug Administration) to retest all valsartan API and tablets containing ZHP API. However, not all products made with ZHP valsartan were found to contain the NDEA impurity (1).

Valsartan is an angiotensin II receptor blocker (ARB) used for the treatment of hypertension. The other ARBs were also found to contain impurities NDMA and NDEA resulting in worldwide recalls. For example, low levels of NDEA were found in losartan tablets manufactured by Hetero Labs India and irbesartan produced by Aurobindo Pharma. In the case of Aurobindo Pharma, the certificate of suitability (CEP) was suspended (2). Of high concern are the ARBs with similar chemical structure containing tetrazole ring such as losartan, candesartan, irbesartan, valsartan and olmesartan (hereafter referred to as sartans) (1).

The purpose of this article is to identify a potential gap in current pharmaceutical industry practice that might have led to the NMDA and NDEA impurities escaping the attention of drug manufacturers and FDA. Integration of ICH Q3A

Guest Editors: Ajaz S. Hussain, Kenneth Morris, and Vadim J. Gurvich

[1] Zeino Pharma FZ LLC, 703-HQ Complex-North Tower, Dubai Science Park, Dubai, UAE.
[2] RAK College of Pharmaceutical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE.
[3] Neopharma LLC, Industrial City of Abu Dhabi (ICAD), Mussafah, Abu Dhabi, UAE.
[4] Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science Center, Texas A&M University, 310 Reynolds Medical Building, Suite 159, College Station, Texas 77843-1114, USA.
[5] To whom correspondence should be addressed. (e–mail: rahman@pharmacy.tamhsc.edu)

guidance document and FDA guidance on genotoxic and carcinogenic impurities in drug substances and products is proposed that would ensure that a risk-based assessment of the chemical synthetic process is performed to anticipate the presence of potential toxic impurities prior to comparing the impurities with their threshold limits.

POTENTIAL PATHWAY FOR NDMA AND NDEA IMPURITIES GENERATION

NDMA and NDEA are compounds in the nitrosamine group which are classified as potential human carcinogens. Their generation in recalled valsartan and other ARBs is linked to the specific manufacturing process adopted by ZHP. ZHP is believed to have made changes to the manufacturing process in 2012 to reduce waste and improve product yield. ZHP changed the synthetic process of tetrazole ring formation in the valsartan molecule. Tributylin azide was replaced with a more toxic compound *viz.* anhydrous sodium azide, and dimethyl formamide was used as solvent (3). Sodium nitrite was subsequently used to quench excess sodium azide following synthesis. In the acidic environment, sodium nitrite forms nitrous acid, which could react with dimethylamine present in dimethylformamide (solvent used in tetrazole forming reaction) to generate NDMA. Similarly, a specific set of reactants and reaction conditions lead to the formation of NDEA impurity (Fig. 1). Tetrazole a common structural feature among ARBs resulted in widespread contamination with impurities in compounds of this class such as losartan and irbesartan.

RISK TO PATIENTS

The European Medicines Agency (EMA) determined the life-time risk of cancer development as 1 in 5000 in patients who had taken an affected valsartan medicine at the highest dose of 320 mg every day from July 2012 to July 2018. The risk assessment considered the levels of NDMA in ZHP's valsartan API since 2012 when the company changed the manufacturing process for valsartan. Further, risk assessment assumed that all the NDMA is transferred to the final product (4). Pottegard et al. performed an expedited assessment of cancer risk linked to NDMA-contaminated product exposure in 5150 Danish patients who were using valsartan and had no history of cancer. The patients were followed for an average period of 4.6 years only. The hazard ratio for overall cancer was 1.09 implying low short-term risk (5). Likewise, the FDA scientists estimated that if patients would have taken a maximum dose of 320 mg of valsartan daily for 4 years, the chances of development of an additional case of cancer is 1 in

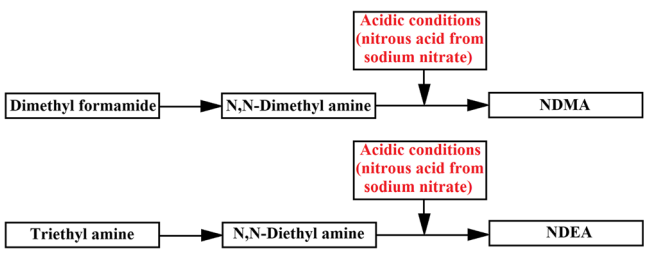

Fig. 1. Potential pathway for generation of NDMA and NDEA impurities

8000. Nonetheless, the presence of NDMA which is widely used in cancer research in pharmaceutical products raises questions and concerns.

The International Agency for Research on Cancer (IARC) has categorised NDMA as a probable carcinogen to humans (6). The pathway for carcinogenic activity is biotransformation to methyldiazonium ion by liver microsomal enzymes, which in turn form DNA adducts such as O^6-methylguanine, a probable carcinogenic agent. A positive relationship between exposure-response has been observed for the intake of NDMA and gastric and lung cancer (7–11). Knekt et al. also observed a positive correlation between NDMA intake and subsequent occurrence of colorectal cancer with a relative risk of 2.12 in a population-based cohort study of 9985 adult Finnish men and women with a follow-up period of 24 years (12).

NDEA is considered as one of the most potent liver carcinogens among nitrosamines. Like NDMA, NDEA is biotransformed to form an ethyl diazonium ion by cytochrome p450 enzymes (mainly CYP2E1). Ethyl diazonium ion can react with nucleophilic sites of DNA and form adducts which can induce cancer (13–15).

ACCEPTABLE DAILY INTAKE

The Integrated Risk Information System (IRIS) established that at a concentration of 7×10^{-4} μg/L of NDMA in drinking two litres of water per day would result in a one in one million lifetime (10^{-6}) risk of cancer in 70 kg human (16). On the other hand, WHO suggests that 0.1 μg/L of NDMA in drinking water would be associated with 10^{-5} cancer risk (17). The USFDA has established action levels of 5 μg/L and 0.01 μg/g in malt beverages and barley malt, respectively of NDMA (18,19). Based on a daily exposure to NDMA or NDEA that results in a 1:100,000 cancer risk after 70 years exposure, FDA has established interim acceptable daily intake (ADI) limits for these impurities in sartans (1) (Fig. 2).

ARE CURRENT CONTROLS ADEQUATE?

The ICH Q3 A (R2) guidance document states, "*The applicant should summarise the actual and potential impurities most likely to arise during the synthesis, purification, and storage of the new drug substance. This summary should be based on sound scientific appraisal of the chemical reactions involved in the synthesis, impurities associated with raw materials that could contribute to the impurity profile of the new drug substance, and possible degradation products. This discussion can be limited to those impurities that might reasonably be expected based on knowledge of the chemical reactions and conditions involved*" (20). The guidance explicitly states that impurities should be summarised based on the knowledge of chemical reactions and conditions involved. According to the Environmental Protection Agency technical fact sheet, NDMA can be generated in reactions involving nitrogen oxides, nitrous acid or nitrite salts. Further, secondary, tertiary and quaternary amines serve as precursors for nitrosamine generation (21,22). Considering the available information, use of dimethyl formamide (a source of dimethyl amine) and sodium nitrite should raise an alarm owing to the high possibility of generating nitrosamines in a reaction. Yet,

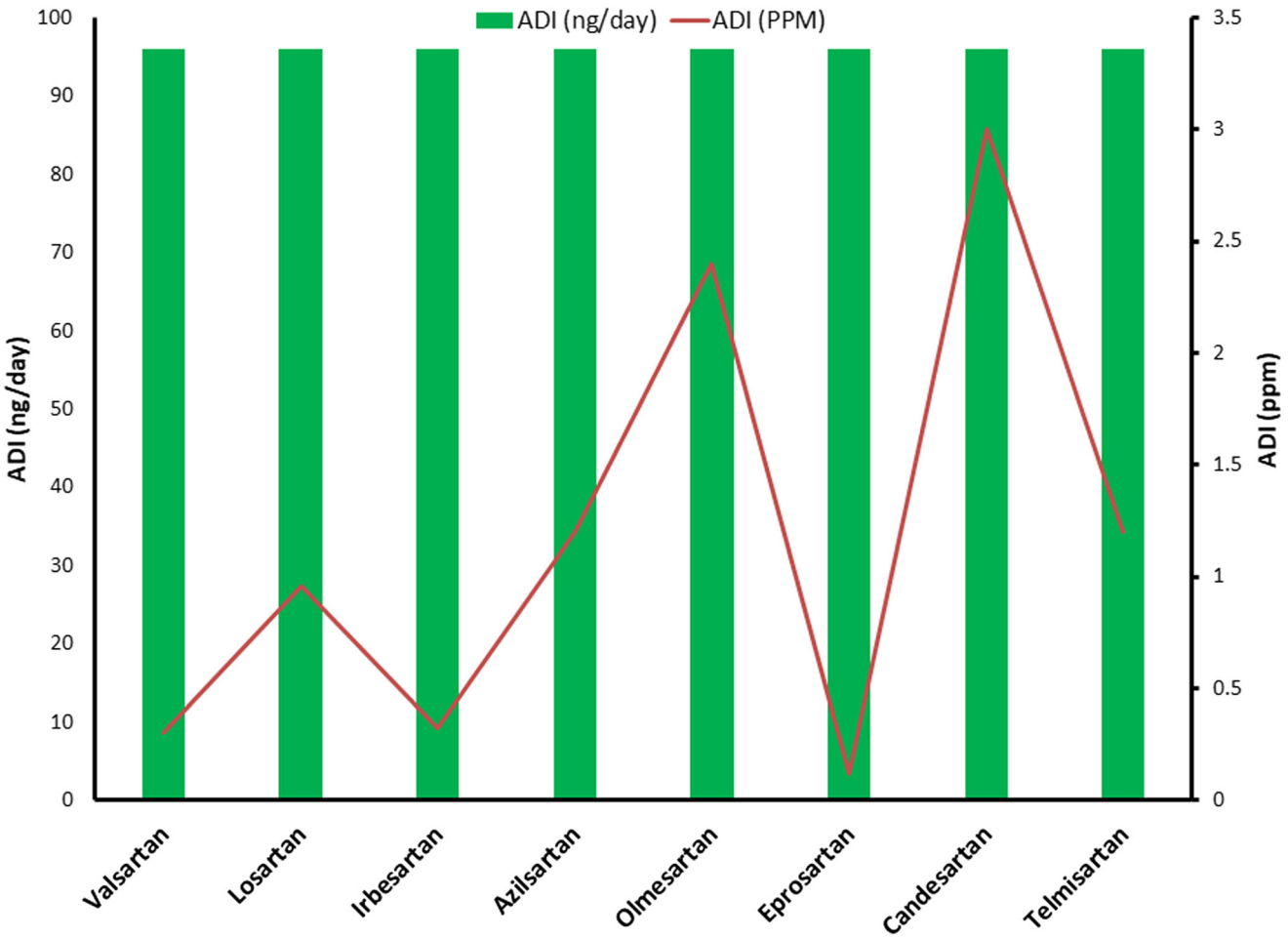

Fig. 2. Limits for NDMA and NDEA. Key: ADI values in (ng/day) are based on daily exposure to a compound such as NDMA or NDEA that results in a 1:100,000 cancer risk after 70 years exposure. ADI values in ppm are based on a drug's maximum daily dose

the manufacturer of recalled sartan API seems to have been oblivious to well-known chemical reactions of this type.

The manufacturer of recalled sartans observed quality management practices currently required by cGMP and previous regulatory audits of the manufacturer's facility were concluded successfully without quality or cGMP shortcomings. The process change that is believed to have resulted in the generation of nitrosamines was also approved through normal regulatory procedures by FDA and European Directorate for the Quality of Medicines (EDQM). It was only after the impurities were detected in commercial batches of valsartan and other sartans that the FDA, on 29 November 2018, issued a warning letter to ZHP citing significant deviations from the cGMP for API (1). The warning letter cited a customer complaint that was received by ZHP regarding detection of NDMA impurity during residual solvent testing for valsartan API. In response to the complaint, the ZHP's investigation was found inadequate and failed to resolve and control the presence of NDMA in valsartan API. Further, FDA determined that the investigation conducted by ZHP lacked a comprehensive analysis of all raw material used during the manufacturing process of valsartan. The impact of process change was not adequately assessed and the potential for generation of mutagenic or other toxic impurities was not considered. This underscores the fact that much emphasis is currently placed on controlling the related substance above and below the reporting and identification thresholds, respectively. The ICH Q3A guidelines mandate reporting threshold of 0.05% for a drug with a maximum daily dose ≤ 2 g/day, whereas the reporting threshold of 0.03% is recommended for a drug substance with a maximum daily dose > 2 g/day (20). Valsartan and most other sartans dosed at ≤ 2 g/day would have a reporting threshold of 0.05%. Even though the FDA limits are stringent for carcinogenic or genotoxic impurities, the sartan incident has revealed that relying solely on reporting threshold criteria (attachment 3 of ICH Q3) is extremely risky. The fact of the matter is that the product in spite of being manufactured according to the current regulatory requirements was contaminated with carcinogenic impurities has reaffirmed that the focus should also include consideration of safety of chemical processes. In fact, the safety and risk assessment of chemical synthesis should be performed much before the impurities are compared to guidance limits. This assumes great importance as there is a high probability that conventional testing and pharmacopeial test methods may not detect all impurities. The importance of a thorough and stringent review by both manufacturers and regulators to assess the potential risks underlying the manufacturing process cannot be overemphasised.

Another pertinent issue is that ICH guidance document suggests that the identification of impurities present at not more than (≤) the identification threshold is generally not considered necessary. Toxic impurities even if present at levels below the identification threshold may still be above their ADI levels, raising serious questions about this statement, even though the guidance document does suggest that the analytical procedures should be developed for these toxic potential impurities at a level not more than (≤) the identification threshold. The Guidance document recognises that, "*For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation/detection limit of the analytical procedures should be commensurate with the level at which the impurities should be controlled*". The notes to attachment 3 also suggest that lower thresholds may be appropriate if the impurity is unusually toxic. However, the reporting threshold limits which are currently used as a bench mark by the pharmaceutical industry seem to have taken precedence over the importance of knowledge and understanding of the chemical synthetic processes. It will serve the interests of the pharmaceutical industry and patients alike if a decision tree (attachment 3 of ICH Q3) begins with a question whether the synthetic process has been comprehensively evaluated for the potential to generate toxic impurities (Fig. 3). The ICH decision tree recommends actions based on the identification threshold. If the impurity is below the identification threshold, no action is required. Certain toxic impurities such as NDMA and NDEA would need to be controlled at much lower threshold levels. For instance, considering NDMA and NDEA are present at below reporting threshold (0.04%), the calculated total daily intake in mg would be much higher than their interim acceptable daily intake limits provided by FDA (Table I). The FDA guidance on genotoxic and carcinogenic impurities in drug substances and products recognises that ICH Q3A and Q3B may allow a genotoxic or carcinogenic impurity to be present in drug product at a level leading to exposure up to 3000 μg/day without requiring identification of the impurity (23).

To reduce the potential lifetime cancer risk associated with exposure to genotoxic and carcinogenic impurities, one of the approaches recommended in the EMA guidance document and FDA guidance on genotoxic and carcinogenic impurities in drug substances and products is to allow only a maximum daily exposure target of 1.5 μg/day. The guidance documents further recommend that if structural alerts suggest high genotoxic and carcinogenic potential, then a maximum daily exposure of 1.5 μg/day would not be appropriate and these impurities should be dealt with on a case-by-case basis. The threshold (1.5 μg/day) corresponds to a lifetime cancer risk of 10^{-5}. Further, the FDA guidance recommends that if an impurity with genotoxic or carcinogenic potential is identified or such an impurity may be expected based on the synthetic route, steps must be taken to address safety concerns associated with these impurities. Below qualification threshold, identified impurities do not need to be qualified (20). Notwithstanding this, identified impurities should be evaluated for genotoxicity and carcinogenicity based on structural activity relationship assessments even if impurity levels are below the qualification threshold. The guidance notes that the impurities with structural alerts for potential genotoxicity may be controlled at a threshold of 0.15 μg/day, which would correspond to a lifetime cancer risk of 10^{-6} (23). However, compounds such as N-nitroso have very high carcinogenic potency and are excluded from the threshold approach in both EMEA and FDA documents (23,24).

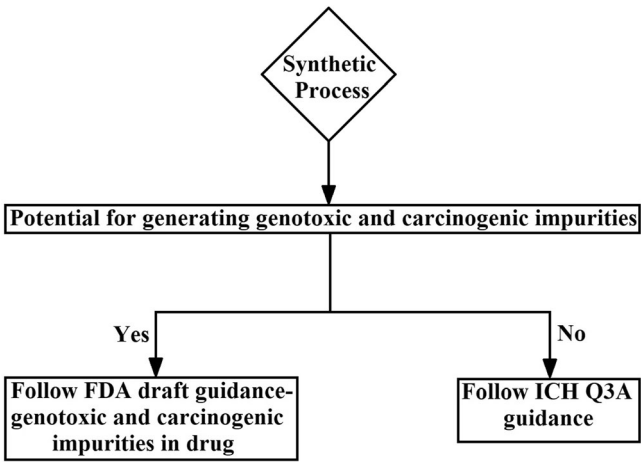

Fig. 3. Decision tree for evaluation of potential carcinogenic and genotoxic impurities

Fortunately, the advancements in chromatographic estimation make it possible to quantify low levels of impurities. The Office of Testing and Research (OTR) has developed a gas chromatographic-mass spectrometric-based method for the detection and quantification of NDMA and NDEA simultaneously in both drug substances and drug products. The General European Official Medicines Control Laboratories Network (GEON) has also developed three methods for detecting these impurities (25,26).

The NMDA and NDEA impurities in sartan brings quality by design (QbD) to the forefront of efforts to address the presence of toxic impurities in API. Identification of critical material attributes and critical process attributes and how they interact is paramount in process understanding. Critical quality attributes (CQAs) derived from quality target product profile (QTPP) link patient safety and medication efficacy to drug development program. During subsequent risk assessment, risk to CQAs due to process components is analysed. Process design using design of experiments (DOE) would unravel information on potential interactions between process components. The design space for critical process components and overall control strategy would ensure that CQAs are met. Therefore, only synthetic routes that would not generate toxic impurities or process conditions that would minimise the generation of impurities would be used for commercial synthetic processes. For instance, Looker et al. used QbD and risk assessment principles to determine the criticality of the impurities and minimise their presence in the product (27). The reduction of nitroaromatic groups to aniline derivative carries high potential of generating genotoxic impurities such as nitroso derivative and hydroxylamine. The authors used *in silico* methods such as Deductive Estimation of Risk from Existing Knowledge (DEREK) and toxicology data to assess the genotoxic potential of the compounds involved in the synthesis. DEREK raised the structural alert for the four compounds (nitroaromatic, aniline derivative, nitroso derivative and hydroxylamine).

Table I. TDI Values Based on Theoretical Result

Sartan	Maximum daily dose (mg/day)	Theoretical result (%)	Calculated total daily intake (TDI) of the impurity (mg)	Acceptable daily intake (NDMA) (ng/day)	Acceptable daily intake (NDEA) (ng/day)
Valsartan	320	0.04	0.128	96	26.5
Losartan	100	0.04	0.04	96	26.5
Irbesartan	300	0.04	0.12	96	26.5
Azilsartan	80	0.04	0.032	96	26.5
Olmesartan	40	0.04	0.016	96	26.5
Eprosartan	800	0.04	0.32	96	26.5
Candesartan	32	0.04	0.0128	96	26.5
Telmisartan	80	0.04	0.032	96	26.5

However, subsequent Ames test showed negative genotoxicity for nitroaromatic and aniline derivative. Looker and co-workers optimised the reaction conditions to minimise the presence of these impurities and comply with the established specific thresholds. They performed catalytic reduction of nitroaromatic group to an aniline derivative through a hydrogenation catalysed by Pd/C and subsequently used validated process steps such as filtration, concentration and crystallisation to remove catalyst. The operating ranges of temperature, amount of catalyst and reaction time were identified through design of experiment leading to not only lower impurity levels (less than the threshold of toxicological concern levels) but also a high product yield (27). Similarly, the process understanding developed by using Qbd resulted in robust control strategy that ensured negligible levels of genotoxic impurities and allowed of elimination of testing of the genotoxic impurities in the final drug substance (28).

Process analytical technology (PAT) tools can be used to monitor reactions in real time to gain a better understanding of processing. Reactive/unstable intermediates and critical endpoint determinations can be improved using real-time reaction monitoring. Multiple PAT tools can be used simultaneously to thoroughly screen the process and determine interactions between various reaction components. Downstream unit processes are crucial in quenching unreacted reagents and removing reaction by-products and impurities especially in situations where API crystallisation does not result in a product completely free from impurities. PAT tools can be used to monitor parameters that are necessary for compliance to critical quality attributes and generation of an appropriate yield for the process.

CONCLUSION

In conclusion, a combination of approaches is required to address the presence of toxic impurities in APIs. The safety and risk associated with a chemical synthetic process should be thoroughly evaluated and understood. ICH Q3A and FDA guidance on genotoxic and carcinogenic impurities in drug substances and products need to be integrated so that the ICH Q3 decision tree (attachment 3) commences with checking of the synthetic process and whether it has been evaluated for the potential to generate toxic impurities. Compliance with ICH Q3 limits should be performed only after the process has been determined to be safe without the risk of generating mutagenic and carcinogenic impurities. Linking product and process development to patient safety and efficacy in QbD paradigm would ensure toxic impurities are either not generated or their level in the product is maintained below acceptable daily intake levels.

ACKNOWLEDGEMENTS

This article reflects the scientific opinion of the authors and not necessarily the policies of the Zeino Pharma FZ LLC and Neopharma LLC.

REFERENCES

1. FDA (2019) FDA updates on angiotensin II receptor blocker (ARB) recalls including valsartan, losartan and irbesartan. Accessed 21 Jan 2019. https://www.fda.gov/Drugs/DrugSafety/ucm613916.htm.
2. EMA (2018) EU authorities take further action in ongoing review of sartans: Zheijiang Huahai placed under increased supervision; Aurobindo Pharma stopped from supplying irbesartan to the EU. Accessed 21 Jan 2019. https://www.ema.europa.eu/en/news/eu-authorities-take-further-action-ongoing-review-sartans-zheijiang-huahai-placed-under-increased#_ftn1
3. Shanley A. After Valsartan Recalls, Regulators grapple with nitrosamine contamination in APIs. Pharma Tech 2018. Accessed 21 Jan 2019. http://www.pharmtech.com/after-valsartan-recalls-regulators-grapple-nitrosamine-contamination-apis
4. EMA (2018b) Press release on 13th September. Update on review of valsartan medicines: risk from NDMA remains low, a related substance NDEA also being investigated. Accessed 21 Jan 2019. https://www.ema.europa.eu/en/news/update-review-valsartan-medicines-risk-ndma-remains-low-related-substance-ndea-also-being
5. Pottegard A, Kristensen K, Ernst MT, Johansen NB, Quartorolo P, Hallas J. Use of N-nitrosodimethylamine (NDMA) contaminated valsartan products and risk of cancer: Danish nationwide cohort study. Br Med J. 2018;362:k3851.
6. IARC (1978) Some N-nitroso Compounds: IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. International Agency for Research on Cancer, Lyon, France.
7. González CA, Riboli E, Badosa J, Batiste E, Cardona T, Pita S, et al. Nutritional factors and gastric cancer in Spain. Am J Epidemiol. 1994;139:466–73.
8. La Vecchia C, D'Avanzo B, Airoldi L, Braga C, Decarli A. Nitrosamine intake and gastric cancer. Eur J Cancer Prev. 1995;4:469–74.

9. Pobel D, Riboli E, Cornée J, Hémon B, Guyader M. Nitrosamine, nitrate and nitrite in relation to gastric cancer: a case–control study in Marseille, France. Eur J Epidemiol. 1995;11:67–73.
10. Goodman MT, Hankin JH, Wilkens LR, Kolonel LN. High fat foods and the risk of lung cancer. Epidemiology. 1992;3:288–99.
11. De Stefani E, Deneo-Pellegrini H, Carzoglio JC, Ronco A, Mendilaharsu M. Dietary nitrosodimethylamine and the risk of lung cancer: a case–control study from Uruguay. Cancer Epidemiol Biomarkers Prev. 1196(5):679–82.
12. Knekt P, Järvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Int J Cancer. 1999;80:852–6.
13. Archer MC. Mechanisms of action of N-nitroso compounds. Cancer Surv. 1989;2:241–50.
14. Nakae D, Kobayashi Y, Akai H. Involvement of 8-hydroxyguanine formation in the initiation of rat liver carcinogenesis by low dose levels of N-nitrosodiethylamine. Cancer Res. 1997;57(7):1281–7.
15. Dragan YP, Hully JR, Nakamura J, Mass MJ, Swenberg JA, Pitot HC. Biochemical events during initiation of rat hepatocarcinogenesis. Carcinogenesis. 1994;15(7):1451–8.
16. EPA Integrated Risk Information System (IRIS). "N-nitrosodimethylamine; CASRN 1993; 62–75-9." https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0045_summary.pdf Accessed 21 Jan 2019**.**
17. WHO (2008). N-nitrosodimethylamine in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/HSE/AMR/08.03/8). Accessed 21 Jan 2019. https://www.who.int/water_sanitation_health/dwq/chemicals/ndmasummary_2ndadd.pdf
18. FDA (1996) FDA compliance policy guides manual: sec. 510.600 dimethylnitrosamine in malt beverages. US Food and Drug Administration, Washington, DC.
19. FDA (1995) FDA compliance policy guides manual: sec. 578.500 dimethylnitrosamine in barley malt. US Food and Drug Administration, Washington, DC.
20. ICH (2006) IMPURITIES IN NEW DRUG SUBSTANCES Q3A(R2). Accessed 21 Jan 2019. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2__Guideline.pdf
21. Krasner SW, Mitch WA, McCurry DL, Hanigan D, Westerhoff P. Formation, precursors, control, and occurrence of nitrosamines in drinking water: a review. Water Res. 2013;47(13):4433–50.
22. Mitch WA, Sedlak DL. Characterization and fate of N-nitrosodimethylamine precursors in municipal wastewater treatment plants. Environ Sci Technol. 2004;38(5):1445–54.
23. FDA (2008) Genotoxic and carcinogenic impurities in drug substances and products: recommended approaches. **Draft guidance**. Accessed 21 Jan 2019. http://www.triphasepharmasolutions.com/Resources/Guidance%20for%20Industry%20Genotoxic%20Agents.pdf
24. EMA (2006) Guideline on the limits of genotoxic impurities. Accessed on Jan 21, 2019. https://www.ema.europa.eu/documents/scientific-guideline/guideline-limits-genotoxic-impurities_en.pdf
25. FDA (2019) Combined N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) impurity assay by GC/MS-headspace. Available at: https://www.fda.gov/downloads/Drugs/DrugSafety/UCM623198.pdf (Accessed 6th March 2019).
26. EDQM (2018) OMCLs release three methods for determination of NDMA in sartans. **Available at: https://www.edqm.eu/en/news/omcls-release-three-methods-determination-ndma-sartans. (Accessed 6th March 2019).**
27. Looker AR, Ryan MP, Neubert-Langille BJ, Naji R. Risk assessment of potentially genotoxic impurities within the framework of quality by design. Org Process Res Dev. 2010;14:1032–6.
28. Cimarosti Z, Bravo F, Stonestreet P, Tinazzi F, Vecchi O, Camurri G. Application of quality by design principles to support development of a control strategy for the control of genotoxic impurities in the manufacturing process of a drug substance. Org Process Res Dev. 2010;14:993–8.