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RESEARCH ARTICLE

International Regulatory Collaboration on the Analysis of Nitrosamines in Metformin-Containing Medicines

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Abstract

Recalls of some batches of metformin have occurred due to the detection of *N*-nitrosodimethylamine (NDMA) in amounts above the acceptable intake (AI) of 96 ng per day. Prior to the recalls, an international regulatory laboratory network had been monitoring drugs for nitrosamine impurities with each laboratory independently developing and validating multiple analytical procedures to detect and measure nitrosamines in metformin drugs used in their jurisdictions. Here, we provide an overview of the analysis of metformin active pharmaceutical ingredients (APIs) and drug products with 1090 samples (875 finished dosage forms (FDFs) and 215 API samples) tested beginning in November of 2019 through July of 2020. Samples were obtained internationally by a variety of approaches, including purchased, received from

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firms via information requests or selected by regional regulatory authorities (either at wholesalers or during GMP inspections). Only one nitrosamine (NDMA) was detected and was only present in some batches of metformin products. For API samples, 213 out of 215 lots tested had no measurable level of NDMA. For FDF samples tested, the number of batches with NDMA above the AI amount for patient safety was 17.8% (156/875). Based on these data, although the presence of NDMA was of concern, 82.2% of the samples of metformin drug products tested met quality and safety standards for patients. Regulatory agencies continue to collaborate extensively and work with marketing authorization holders to understand root causes of nitrosamine formation and agree on corrective actions to mitigate the presence of NDMA in future metformin batches.

KEY WORDS drug supply · International regulatory laboratories · metformin · NDMA · nitrosamines

INTRODUCTION

Metformin is widely used alone or in combination with other medicines to treat type 2 diabetes [1] and is usually a first-line treatment whose mechanism of action is to reduce the production of glucose in the body and reduce glucose absorption from the gut. Metformin can be prescribed as a maintenance drug for years and is the only oral medication approved for diabetes treatment in children [2]. Metformin is considered to be an essential medicine according to the World Health Organization (WHO) [3] and as such, any decision to recall or prevent release of batches due to quality issues needs to be balanced against the detrimental health effects of diabetes patients not receiving their medicines.

The drug is available as a solution or suspension but is typically prescribed as a solid oral dosage form with 250 to 1000 mg of metformin in an immediate release or extended-release formulation or as a combination product (*i.e.*, containing another fixed dose active pharmaceutical ingredient (API) in addition to metformin). In late 2019, there were reports of the presence of *N*-nitrosodimethylamine (NDMA) in metformin products [4–6].

NDMA is classified as a probable human carcinogen (a substance that could cause cancer) on the basis of animal studies [7]. Nitrosamines are known impurities, present in parts-per-million (ppm or ng/mg) to parts-per-billion (ppb or pg/mg) amounts in foods, beverages, cosmetics, water, tobacco products and consumer goods [8–11]. In the 1970s, Lijinsky and coworkers reported the potential for drugs to react with nitrites to form nitrosamines like NDMA *in vivo* after the drugs were taken by patients [12, 13].

As reviewed by Parr and Joseph in 2019, there were also measurements from the 1970s and 1980s on nitrosamine amounts in drugs themselves [14]. However, the results from these early studies varied greatly across products and batches of drugs (*e.g.*, [15–18]). Importantly, as noted by the authors of these early studies (which required multiple extraction and concentration steps), the measurements were prone to analytical artifacts [15–18]. In addition, even more recent nitrosamine reports have observed and corrected for potential analytical artifacts in the validation of analytical procedures for nitrosamine quantification (*e.g.*, [19–21]) highlighting the care needed in performing such measurements.

In 2018, observations of NDMA and other nitrosamines in angiotensin receptor blocker (ARB) drugs led to recalls of batches of products which had unacceptable amounts of nitrosamines [14, 22, 23]. These events led to increased awareness of the risk of nitrosamines being present in drugs and, subsequently, other drugs have been found to contain nitrosamines with different root causes and sources of nitrosamine impurities [24]. Overall, only since the valsartan contamination has there been increased risk assessment for and observation of nitrosamines as impurities in pharmaceuticals using current analytical technology.

In response, regulatory guidance has been published to help manufacturers mitigate nitrosamines in future drug batches while maintaining an adequate supply of medication to patients [25, 26]. Furthermore, pharmaceutical regulatory laboratories have published examples of analytical procedures to detect and quantify nitrosamines in specific drugs to speed the risk-based screening of manufacturing processes for nitrosamines (FDA.gov, EDQM, and HSA websites [27–31]). Of note, the 2019 Parr and Joseph review of analytical procedures for the detection of nitrosamines in pharmaceuticals highlighted the necessity to combine chromatographic separation techniques with highly sensitive detection methods for determining trace nitrosamine amounts in drugs [14].

To date, the only nitrosamine detected in metformin products is NDMA [32]. As noted by Fritzsche *et al.* or Jires *et al.*, dimethylamine (DMA) is a known impurity associated with metformin drugs and DMA in the presence of nitrite or organic peroxides under certain conditions can lead to the formation of NDMA [19, 33, 34]. The acceptable intake (AI) is an amount of NDMA per maximum daily dose (MDD) of API (*e.g.*, 96 ng per day/3000 mg of metformin IR or 32 ppb (pg/mg)) that is deemed acceptable for patient safety. In the EU, a frequently authorized MDD is 3000 mg for metformin drugs. By contrast, in the USA, the immediate release product has an AI of 38 ppb (MDD of 2550 mg of API) and the extended-release product has an AI of 48 ppb (MDD of 2000 mg of API). As the amount of NDMA associated with the API will vary with dosage forms, the ppb limit can be used independent of dosage form to calculate potential exposure to NDMA by a patient taking different doses (*e.g.*, 1000 mg times 32 ppb yields 32 ng of NDMA

in an immediate release product relative to the 96 ng per day amount).

The metformin MDDs are high compared to the MDD of most other drugs and that makes the relative amount of NDMA (in ppb) in metformin commensurately lower to meet the 96 ng/day AI threshold. As noted above, procedures to measure ppb amounts of nitrosamines and their associated analytical issues have been reported in the scientific literature, thus, for metformin or other high dose (> 1 g) medicines, application of sufficiently sensitive and specific analytical procedures may not be straightforward in all pharmaceutical quality control laboratories operating under good manufacturing process (GMP) or current GMP (cGMP) guidelines. However, all the regulatory agency laboratories in this study were able to rapidly perform these analyses with available equipment. The AI amounts were the analytical targets used by laboratories to develop and validate analytical procedures (as per ICH Q2(R1)) with appropriate performance characteristics (*i.e.*, accuracy, sensitivity, and specificity) for measuring trace amounts (ppb) of NDMA in metformin.

In 2020, metformin batches on the US market were reported in medRxiv (a non-peer reviewed preprint journal) to have amounts of NDMA above the AI threshold by a private laboratory, with 42% of 128 metformin drug product lots tested having detectable NDMA amounts and, when scaled to maximum daily dose, 36% had NDMA amounts over the allowable intake amount using one method [35]. Subsequently, applying orthogonal methods, the US FDA discovered that the test method used by the private laboratory was flawed and led to over-reporting of NDMA amounts [21], yet the FDA result did confirm that NDMA had been found in some products albeit with fewer products above the AI (8 of 38 or 21% [21]). In addition, a report from the Polish OMCL laboratory by Zmyslowski *et al.* showed 33% of 105 metformin samples tested had NDMA above the AI amount [20].

These varying reports were of concern as an accurate assessment of metformin quality is an important public health issue. Medicines are intended to improve patients' health so the presence of genotoxic and carcinogenic impurities above certain levels was and is unacceptable. Patients may not take lifesaving medication if there is a perception of "widespread" contamination, even if their particular medication is not affected. The current study provides a snapshot of the extent of contamination of metformin medicines at a time when, globally, regulators and regulated industry are working to investigate the amount and source of nitrosamine impurities present in metformin medicines and implement appropriate corrective measures.

Constantly sharing information regarding testing, the LC–MS or GC–MS technology-based analytical procedures used by the regulatory laboratories were developed independently and validated for use in each location as part of a rapid response to concerns about nitrosamine impurities in metformin. Thus, while the analytical procedures used may be similar in a broader technological view, they differed in the details of the approach used at each location. The work presented here has been made possible by unprecedented international regulatory collaboration and information sharing on the nitrosamine topic.

METHODS AND MATERIALS

The laboratories reporting results for the current analysis were the Official Medicines Control Laboratories (OMCLs) from the General European OMCL network (from Germany, Poland, Switzerland, and the Netherlands), Therapeutic Good Administration (TGA, Australia), Health Sciences Authority (HSA, Singapore) and the United States Food and Drug Administration (US FDA). In total, 1090 samples were obtained from API manufacturers and finished dosage form manufacturers providing products to different regions. Depending on the region, 4 to 11 API producers supplied the 3 to 32 finished dosage form (FDF) manufactures that provided samples for regulatory laboratory testing. Overall, the studies covered 13 unique API producers and about 90 unique producers of finished dosage forms worldwide. Analytical procedures for the intended purpose of nitrosamine impurity assessment in metformin APIs or FDFs containing metformin were developed mostly independently by the regional laboratories. While details of the development and validation of these procedures will not be provided here as they are available from other sources (see below), one important aspect of measurements of trace amounts (ppb (pg/mg)) of impurities are the detection and quantification limits (usually abbreviated as DL or LOD and QL or LOQ). DL and QL are important analytical performance characteristics in the present case because they establish thresholds for the "absence" of a nitrosamine (based on method DL) and a threshold above which the amount measured provides accuracy and precision sufficient for quantification purposes (QL). Table I shows the DLs and QLs for the analytical procedures used for the present work.

Additional information about some of the analytical procedures can be found on the websites of the EDQM, HSA and the US FDA [27–31] or the published literature for the Polish OMCL laboratory [20] and US FDA [21] (details are provided in Supplemental Table 1). Many of the laboratories use a direct extraction of the respective drug substance or finish dosage form with a subsequent dilution and filtration step. Afterwards, the nitrosamines present in the extracted supernatants are measured via direct injection (DI) on GC–MS or LC–MS instruments. These short workup procedures were chosen to minimize any potential nitrosamine

Table I An Overview of the Sensitivities of the Analytical Procedures Developed and Validated by Ten Laboratories for the Metformin Products Tested in This Study. The QLs and DLs Are Given in ppb Relative to Amounts of Drug Substance

Type of analytical procedure	Analytical procedure QL/DL (ppb) for NDMA in metformin									
	Canada HC	Singapore HSA	Germany-Bavaria LGL DE-BY	Poland NIL Polish OMCL	Netherlands NL RIVM OMCL	Germany-Bremen OMCL INPHA	Germany-Karlsruhe OMCL BW	Switzerland Swissmedi-cOMCL	USA USFDA	Australia TGA
LC–MS/MS	10/2[##]				30/9[##]		25/15[#,###]			
LC-HRMS									30/10[##]	8/3[##]
GC-HRMS		20/10[###]								
GC/MS			20/6[##]			30/15[##]				
GC–MS/MS	15/10[##]			14/4[###,*] 26/8[###,**] 27/8[###,***]			15.5/6.5[###]	15/5[##]		

[#]Based on visual evaluation.

[##]Based on signal-to-noise.

[###]Based on the standard deviation of the response and the slope.

[*]QL/DL that apply to API.

[**]QL/DL that apply to FDFs.

[***]QL/DL that apply to modified release product.

Table II A Table of Aggregated Metformin API Testing Data from the 10 Participating Laboratories

Aggregate data on metformin APIs	Totals
API samples tested	215
Number of API manufacturers/sources providing samples	13
Samples positive* for NDMA	2
Samples with NDMA values greater than the AI amount (32 ppb**)	0
% API with NDMA values greater than the AI amount (32 ppb)	0%
% API NDMA positive samples	0.9%
Range of NDMA amounts recorded (ppb) in one laboratory***	17 to 21

*The two samples were above the QL of 15.5 ppb for the method used.

**The lowest NDMA AI value from the participating regions was used (based on 96 ng/3000 mg or 32 ppb).

***NDMA positive API values were recorded in one laboratory in 2 out of 110 samples.

Table III A Table of Aggregated Metformin FDF Testing Data from the 10 Participating Laboratories*

Aggregate data on metformin FDFs	Totals
Finished dosage form (FDF) samples tested	875
Approximate number of FDF manufacturers providing samples	90
Samples positive** for NDMA	335
Samples with NDMA greater than the AI amount (32 ppb***)	156
NDMA positive FDF samples (above the analytical procedure detection limit (DL)) but below the measurement quantification limit (LOQ) w/o numerical values recorded	54
Number of NDMA positive FDF samples with numerical values recorded	281
% FDF with NDMA values greater than the AI amount (32 ppb)	17.8%
% FDF NDMA positive samples	38.3%
% FDF samples with an NDMA numerical value recorded	32.1%
Range of NDMA amounts recorded (ppb) across 10 laboratories and 281 samples	8 to 688
Range of average NDMA amounts (ppb) across 10 laboratories and 281 samples	24 to 77
Range of median NDMA amounts (ppb) across 10 laboratories and 281 samples	19 to 75

*The results previously reported from the Polish OMCL are included in the aggregated data presented in the table (20).

**Samples were above the method DL.

***The lowest NDMA AI value from the participating regions was used (96 ng/3000 mg of API) for this purpose.

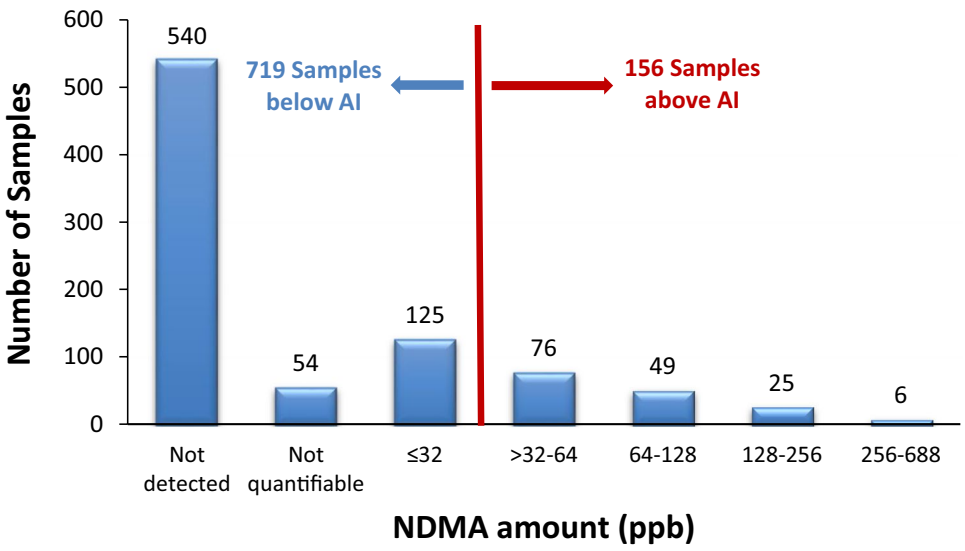

Fig. 1 A distribution of observed NDMA amounts across 875 metformin FDFs tested in this study. The number of samples in each NDMA category is annotated in the plot

loss or formation of nitrosamines during preparation steps prior to introduction of samples into the instrument. In some laboratories, a second orthogonal measurement was developed and validated to verify the accuracy of the results obtained with the primary analytical procedure (*e.g.*, [21]). As a further check of the accuracy of the measured amounts of NDMA reported by 5 of the analytical procedures used, an identical FDF batch was distributed and tested by laboratories in Germany-Bavaria, Germany-Karlsruhe, Poland, and Switzerland. The cross-laboratory test results showed good comparability (average of 137 ppb ± 20 ppb maximum difference) (data not shown).

RESULTS AND DISCUSSION

Tables II and III show a summary of the analysis of samples tested across 10 regulatory labs representing European and North American regions, Singapore, and Australia. Importantly, because the samples received and tested by the 10 regulatory laboratories in this study differed in number and type of sample (*e.g.*, API versus FDF, fixed dose combination product versus metformin only dosage forms, or immediate release versus extended-release formulations), the percentages calculated in Tables II and III are not a comprehensive market survey. Furthermore, in some cases, targeted sampling (collecting more samples from firms that already had positive samples) was used and would likely artificially elevate the percentage of positive samples reported in this analysis. Values were recorded across ten laboratories and used different independently developed and validated analytical procedures. Because of the variation in sample type and number across regions, aggregated data are presented for the samples reported in this work.

The data in Table III show that 335 out of 875 FDF samples had NDMA concentrations above DL. Out of these 335 NDMA positive samples, numerical values (partially below QL) were provided for 281 samples. The distribution of the not detected, not quantifiable, below AI and above AI amount samples are shown in Fig. 1.

A primary outcome of the intensive sampling of APIs and FDFs was that NDMA was for the most part absent from the drug substance (Table II). For metformin, there were only two low positive API samples recorded in these studies (at 17 and 21 ppb using a method with a QL of 15.5 ppb) out of 215 APIs tested. The API samples were tested with analytical procedures with DLs of 15 ppb or lower and QLs of 30 ppb or lower with most of them (178/215 or 83%) tested with techniques with DLs of 10 ppb or lower and QLs of 20 ppb or lower. The absence of nitrosamine in the majority of metformin APIs differs from the results observed for ARBs (*e.g.*, [36]) where the APIs (and subsequent FDF batches) were found to contain nitrosamines. By contrast, the presence of NDMA was found primarily in formulated metformin products (Table III and Fig. 1). Thus, the process of manufacturing the FDF from the API seems to result in the generation of NDMA in some (but not all) products. The reasons for the presence of NDMA in FDFs from certain manufacturing processes is an area of active investigation.

While many questions as to the exact mechanisms of NDMA formation in metformin FDFs are still unanswered, several potential root causes were identified during the analysis of FDFs or in experimental batches produced by some marketing authorization holders on a laboratory scale. One proposed root cause was based on the presence of dimethylamine (DMA) which is a known by-product of the metformin API synthesis and typically monitored by compendial monograph tests for metformin [37]. Studies of the OMCL Germany-Bavaria showed (data not presented) that DMA levels in metformin drug substance varied between different API manufacturers. Using quantification of DMA by qNMR, DMA was found to range from the QL (< 10 ppm) up to almost 500 ppm (data not shown), which is the limit in the monograph of the European Pharmacopoeia which uses an HPLC method. Under certain conditions, DMA appears to react with nitrite originating from excipients used in metformin products like povidone or hydroxypropylmethylcellulose (hypromellose). For example, in data provided by a FDF manufacturer, nitrite amounts ranged around 2 ppm for povidone and 0.8–1.8 ppm for hypromellose, respectively. The potential for small amounts of reactive impurities, including nitrites, in common excipients has been previously described [38].

Support for the DMA-excipient-nitrite hypothesis was given by initial investigations on experimental immediate release batches that showed that NDMA amounts dropped from higher than AI amounts (maximum: 0.037 ppm) to values lower than DL, when povidone and hypromellose were chosen from different suppliers whose products contained only traces of nitrite (*i.e.*, less than 0.01 ppm), whereas the DMA amounts in the API batches used for the experimental batches remained unchanged. Subsequent analysis by the OMCL Germany-Bavaria verified the success of the corrective actions by the manufacturer to minimize NDMA in their metformin products (data not shown). A synopsis of these results suggests that NDMA levels are potentially influenced by varying levels of nitrite, while in this limited study DMA concentrations seemed to have a minor impact on the associated NDMA concentrations: the NDMA levels in FDFs from one MAH ranged around 28 ppb, whereas DMA levels in different API batches from the unique API supplier used for manufacture of these FDFs ranged from 25 to 218 ppm (data not shown). Similar observations have been reported by Jires and Dousa [34]. An alternate DMA derived NDMA formation mechanism has been proposed where the combination of organic peroxides in povidone excipients and DMA in metformin APIs resulted in NDMA formation [33].

Regardless of the root cause, of the FDFs tested here, 18% contained amounts that were not acceptable (greater than the AI amount) while a similar number of samples (21%) contained detectable NDMA amounts which were acceptable. Thus, in this survey, ~ 80% of the products were safe for patient use. Importantly, the percentages of metformin products meeting regulatory quality requirements in this "snapshot" are likely higher than 80% because of sampling bias of the regulatory laboratories toward manufacturers which already had demonstrated NDMA positive products. Of note, as NDMA amounts measured below QL are considered less reliable for quantitative purposes, in 54 FDF samples, a metformin sample was recorded as positive (above DL but below QL) but no numerical value was attributed to such samples.

As shown in Table I, the analytical procedure QLs ranged from 8 to 30 ppb so these 54 samples had at a maximum less than 30 ppb of NDMA present. As the sample testing was done in parallel across laboratories in the face of a rapidly evolving situation, the result recording practices differed across the laboratories involved, with some recording values for amounts above the detection limits while others recorded values for samples where they observed amounts above the method QL. For those metformin batches which were found to be above the AI amount, some regulatory agencies or manufactures initiated recalls on a case by case basis depending on the local supply situation (*e.g.*, (27)).

For the samples where NDMA could be quantified, the amounts observed across regions varied with a range of mean values from each of the ten laboratories from 24 to 77 ppb (281 samples). The range of median values was 19 to 75 ppb, indicating that the mean values were increased by outliers outside of the range observed in most of the samples. Overall, the range of NDMA amounts for 281 individual samples tested was 8 to 688 ppb across all the FDF samples. Samples from 2 manufacturers (out of 90) exhibited (some) extreme values. When these outliers were removed from the analysis, the range of individual batch values was 8 to 179 ppb.

Unlike the ARBs which were found to contain nitrosamines because of combinations of reagents used in specific synthesis steps in the API manufacturing process [36], NDMA was found primarily in metformin FDFs tested in this study (Table II and Table III). The results of a broad multi-regional screening performed in this work confirmed that while there were FDFs where the AI for NDMA was exceeded, the majority of the metformin products on the market over a 9-month period had either no NDMA impurities present or had amounts of NDMA present that were less than the AI of 96 ng per day when taking the MDD (Table III).

As previously mentioned, there are many potential source of nitrosamines in food, beverages, and consumer products [8]. With the advent of improved analytical procedures to detect and quantify nitrosamines in trace amounts, many industries have made measurements and subsequent adjustments to manufacturing steps to minimize the presence of these impurities. For example, in the 1970s many beers were found to contain 1 to 10 µg of NDMA per kg (1 to 10 ppb) which was formed in the malt-kilning step [39]. Subsequently, a thorough understanding of the root cause allowed brewers to modify their processes and, as a result, most beers now contain no detectable NDMA (*e.g.*, in a 2006 survey, 79% of 138 beers from 42 countries contained no detectable NDMA with only three exceeding 0.1 µg of NDMA per kg (0.1 ppb)) [40]. For human medicines, since the recent discoveries of nitrosamine impurities, many drug regulatory agencies have requested marketing authorization holders of medicinal products to systematically evaluate the risk of presence of nitrosamines in their products, test batches for nitrosamines prior to release and, where needed, to take necessary measures to minimize to safe amounts or eliminate nitrosamines in their products [25, 26, 41].

For metformin drugs, certain batches from specific manufactures reported amounts of NDMA up to 688 ppb while other manufacturers had no detectable NDMA, indicating a connection between the specific manufacturing process conditions used by certain manufacturers and higher amounts of NDMA. This connection is still under active investigation as the subset of samples with NDMA present at greater than the AI amount are a public health concern. Of note, similar averages and ranges to those observed in this study of aggregated data across ten laboratories were reported by the Polish OMCL laboratory for 105 samples which are included in the current analysis (the Polish OMCL laboratory results represent 105/875 or 12% of the data aggregated here) [20].

Finally, in the 1970s and 1980s when there was concern about drugs being nitrosated in humans after taking medication, experiments were performed that showed the hypothetical nitrosation reactions could be inhibited in gastric fluid by the presence of antioxidant compounds like ascorbic acid (vitamin C) or α-tocopherol (vitamin E) [42, 43]. More recently, the inhibition of nitrosamine formation in solid oral dosage forms with antioxidants added to the formulations was demonstrated [44]. In addition, the most common pathway for nitrite driven nitrosation reactions in solution is favored at acidic pH, so adjusting solid oral dosage form formulations to more neutral pHs may also inhibit nitrosamine formation where that mechanism applies. Overall, mitigation of nitrosamine formation in finished dosage forms found to be at risk by formulation changes is a potential solution that could be broadly applied to many drugs [45].

CONCLUSIONS

Here, a snapshot is provided of the amount of NDMA positive medication in the metformin supply of the participating regions over 9 months of testing (November 2019 to July of 2020). The aggregated results indicate that > 80% of the metformin batches taken from the different markets in the first half of 2020 were of appropriate quality for patient use. Given the regional sampling practices employed, where more batches were taken from manufacturers deemed to be at risk of contamination, 80% is potentially an underestimate of acceptable products that were present in the market. Furthermore, these results do not agree with the findings of a private firm which observed that their testing of US market products "reveals widespread contamination" [35]. Notably, the private firm's test method was subsequently found to be flawed [21].

Looking forward, to prevent metformin containing NDMA greater than AI amounts from reaching patients, some regulators have requested that newly manufactured batches of drug product are tested for NDMA before being released to the market. In addition, investigations into the root causes continue [46]. The amount of nitrosamine in the drug supply should be minimized to safe amounts by applying greater product and process knowledge and lessons learned from a range of products which have been found to contain nitrosamines, along with the implementation of mitigation steps in the manufacturing processes. Ultimately, drug substance and drug product manufacturers are responsible for understanding their processes, which includes preventing the presence of unacceptable impurities.

Importantly, global cooperation by regulatory laboratories has allowed rapid development and publication of analytical procedures that can help manufacturers develop and validate their own methods more quickly. Furthermore, regulatory agencies have provided guidance and timelines for manufacturers to minimize nitrosamines in future batches (*e.g.*, FDA Guidance [25], EMA Guidance [26]) and will continue to monitor the market supply to assure availability of medications to patients.

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Author Contribution All of the authors of this study made substantial contributions to the conception or design of the work or the acquisition, analysis or interpretation of data for the work. All authors assisted in drafting the work or revising it critically for important intellectual content. All authors obtained final approval of the version to be published from their regional regulatory authorities. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declarations

Conflict of Interest The authors declare no competing interests.

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