

## Nitroso-Derivatives of $\beta$ -blocker and $\beta$ -agonists

### INTRODUCTION

$\beta$ -blockers, also known as  $\beta$ -adrenergic blocking agents, are medications that reduce blood pressure by blocking norepinephrine and epinephrine from binding to their receptors. The  $\beta$ -blockers are often called “olols” because their names all end with an -olol.

$\beta$ -blockers are used to manage a variety of conditions. They include, but are not limited to cardiac arrhythmias, heart failure, high coronary artery disease risk, diabetes, post heart attack (myocardial infarction), angina pectoris due to coronary atherosclerosis, and hypertension (high blood pressure). (In the management of hypertension, a beta-blocker may be used alone or concomitantly with other antihypertensive agents, particularly thiazide diuretics).

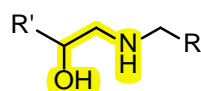
$\beta$ -Adrenergic agonists (also known as  $\beta$ -agonists) are derivatives of adrenaline that bind to  $\beta$ -receptors. They are potent bronchodilators used for the treatment of asthma and obstructive lung disease.

$\beta$ -Blockers and  $\beta$ -agonists have a secondary amine that can undergo nitrosylation under suitable conditions to give *N*-nitrosamine derivatives. **Table 1** shows the structures of the nitrosamines of most marketed  $\beta$ -blockers, divided to 3 groups (as will be discussed below): 1a) *N*-isopropyl (and isopropyl-like) analogs; 2a) *tert*-butyl analogs; and 3)  $\beta$ -blockers with two  $\text{CH}_2$  groups at the  $\alpha$ -positions to the amine.

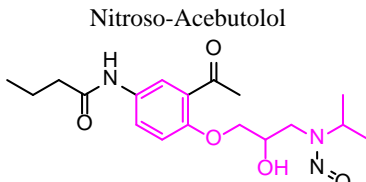
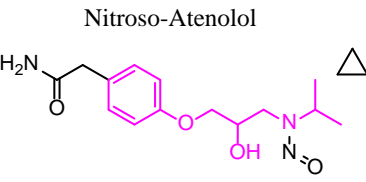
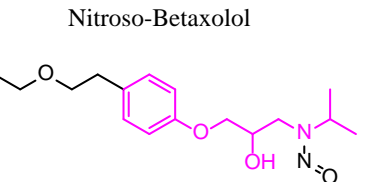
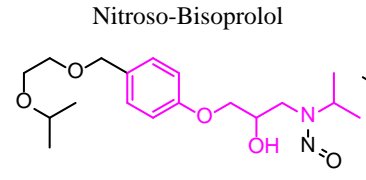
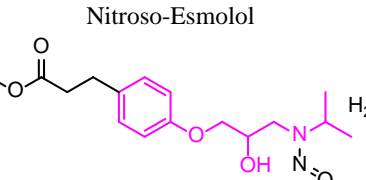
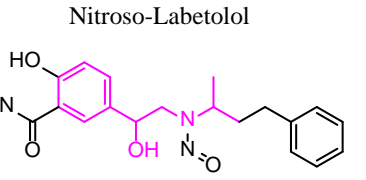
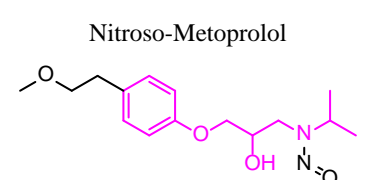
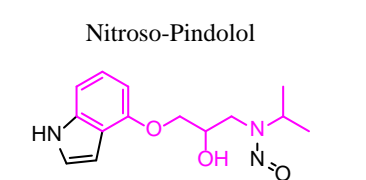
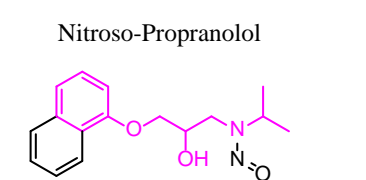
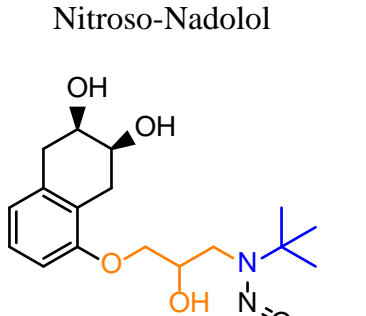
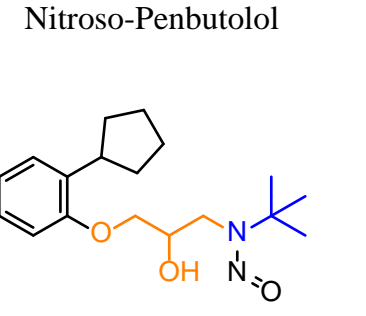
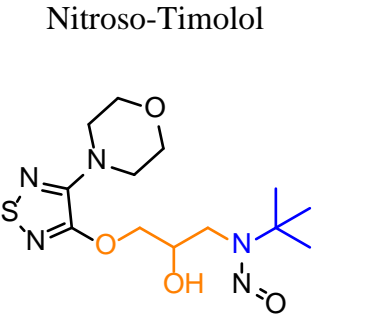
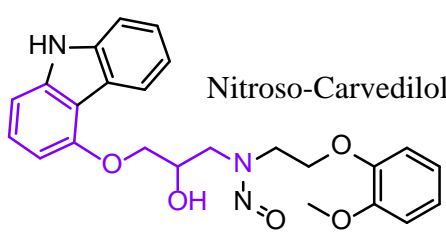
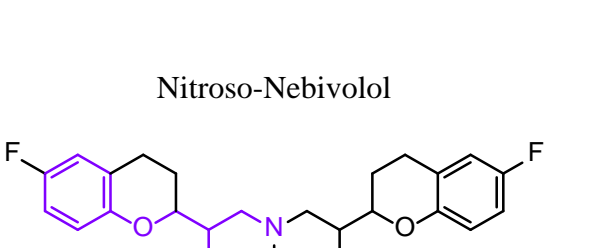
Many of the  $\beta$ -blockers share a larger scaffold of 1-(isopropylamino)-3-phenoxypropan-2-ol (highlighted in magenta in **Table 1**), and others have similar structures, with alkyl/aryl/alkoxy derivatives branching out at the  $\text{R}'$ -position.

Many  $\beta$ -agonists have a similar motif as the  $\beta$ -blockers, where one of the  $\alpha$ -positions has an *N*-isopropyl (or isopropyl-like) group, or a *tert*-butyl group, and the side of the amine has the same  $\beta$ -hydroxy moiety. **Table 2** shows the structures of the nitrosamines of many of the marketed  $\beta$ -agonists, divided to similar groups as described for the  $\beta$ -blockers: 1b) *N*-isopropyl (and isopropyl-like) analogs; and 2b) *tert*-butyl analogs. The common scaffold, 2-amino-1-phenylethanol, found in the  $\beta$ -agonists, is highlighted orange in **Table 2**.

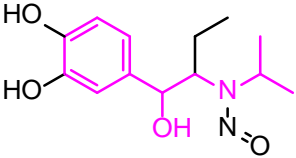

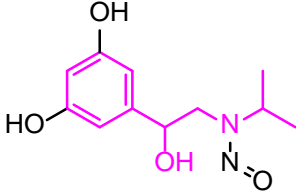
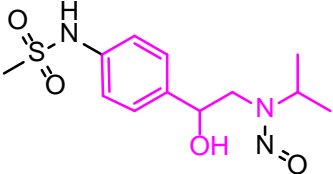
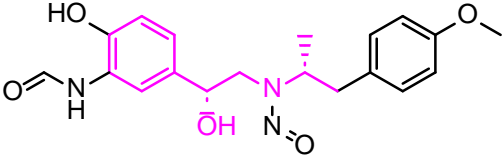
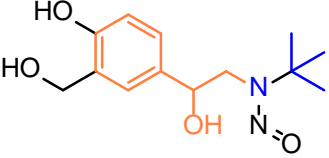
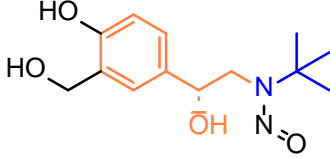
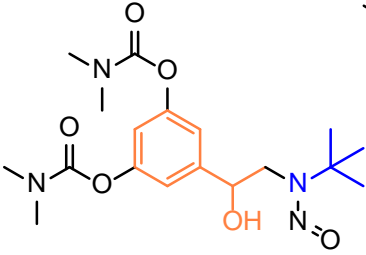
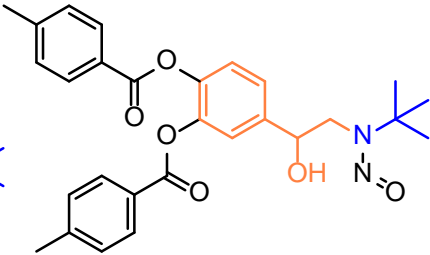
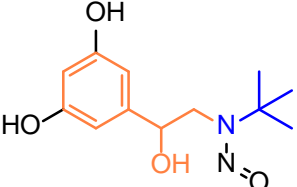
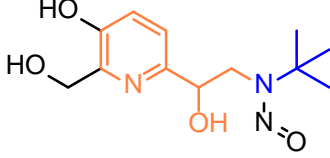
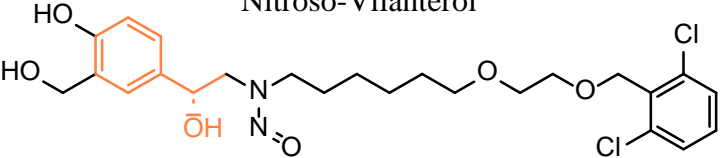
All the  $\beta$ -blockers and  $\beta$ -agonists contain an *N*-alkylethanolamine motif, which can be identified by the hydroxy group at the  $\beta$ -position to the amine (highlighted below in yellow).



**Table 1. Nitrosamines of  $\beta$ -blockers**

<b>Group 1a: <i>N</i>-isopropyl (and isopropyl-like) analogs</b>		
<p>Nitroso-Acebutolol</p> 	<p>Nitroso-Atenolol</p> 	<p>Nitroso-Betaxolol</p> 
<p>Nitroso-Bisoprolol</p> 	<p>Nitroso-Esmolol</p> 	<p>Nitroso-Labetolol</p> 
<p>Nitroso-Metoprolol</p> 	<p>Nitroso-Pindolol</p> 	<p>Nitroso-Propranolol</p> 
<b>Group 2a: <i>tert</i>-Butyl analogs</b>		
<p>Nitroso-Nadolol</p> 	<p>Nitroso-Penbutolol</p> 	<p>Nitroso-Timolol</p> 
<b>Group 3a: <math>\beta</math>-blockers with two CH<sub>2</sub> groups at the <math>\alpha</math>-positions to the amine</b>		
<p>Nitroso-Carvedilol</p> 	<p>Nitroso-Nebivolol</p> 	

**Table 2. Nitrosamines of  $\beta$ -agonists**

<b>Group 1b: <i>N</i>-isopropyl (and isopropyl-like) analogs</b>		
Nitroso-Isoetharine 	Nitroso-Isoprenaline 	Nitroso-Metaproterenol (Nitroso-Orciprenaline) 
Nitroso-Sotalol 	Nitroso-Formoterol 	
<b>Group 2b: <i>tert</i>-Butyl analogs</b>		
Nitroso-Albuterol 	Nitroso-Levalbuterol 	
Nitroso-Bambuterol 	Nitroso-Bitolterol 	
Nitroso-Terbutaline 	Nitroso-Pirbuterol 	
<b>Group 3b: <math>\beta</math>-agonist with two CH<sub>2</sub> groups at the <math>\alpha</math>-positions to the amine</b>		
Nitroso-Vilanterol 		

## ASSESSMENT

### Toxicity of nitrosamines of $\beta$ -blockers and $\beta$ -agonists

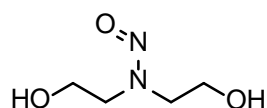
Very little literature data is available for the toxicity of nitrosamines of  $\beta$ -blockers and  $\beta$ -agonists. Martelli et al. (1994)<sup>1</sup> showed that nitroso-propranolol, nitroso-metoprolol, nitroso-nadolol, nitroso-atenolol, and nitroso-sotalol, were markedly less clastogenic than NDMA in an acute rodent liver micronucleus assay. From the *in vivo* micronucleus study conducted by Martelli et al., the authors report a modest increase in micronucleated cells in the rat liver, but no clastogenic effect in bone marrow and spleen. In contrast, NDMA was markedly more clastogenic in liver and also induced micronuclei in the bone marrow and spleen. Although no other *in vivo* genotoxicity/carcinogenicity data was found in the literature for nitrosamines of  $\beta$ -blockers, the Martelli et al. data provides significant evidence that this class of nitrosamines is less potent.

### Structure Activity Relationship (SAR) analysis

A distinct structural element in all the  $\beta$ -blockers and the  $\beta$ -agonists in this discussion is the hydroxy moiety  $\beta$  to the amino group. The structural features of other side of the amine fit into three structural groups: 1) *N*-isopropyl (and isopropyl-like) analogs; 2) *tert*-butyl analogs; and 3) carvedilol, nebivolol and vilanterol that have two  $\text{CH}_2$  groups at the  $\alpha$ -positions to the amine.

#### *$\beta$ -Hydroxy substitution*

The effects of  $\beta$ -hydroxy substitution on nitrosamine toxicity has been described by Ponting et al. (2022)<sup>2</sup> as being associated with both increased and decreased carcinogenic potency in animals, depending on the structural motifs present in the rest of the *N*-nitrosamine molecule. They explain that the decreased carcinogenicity activity is because  $\beta$ -hydroxyl substituents on *N*-alkylnitrosamines lead to increased polarity and potentially disfavor CYP-mediated  $\alpha$ -carbon oxidation, and that the increased activity is because such compounds can undergo enzymatic oxidation on the  $\beta$ -hydroxy group to yield  $\beta$ -carbonyl derivatives which are associated with increased animal carcinogenic potency. However, when comparing potencies of nitrosamines containing a  $\beta$ -hydroxyl to the amine, the simple structure of *N*-nitroso-diethanolamine (NDELA; **Figure 3**), consisting of two  $\beta$ -hydroxy groups is a weak carcinogen with an established acceptable intake (AI) of 1900 ng/day.<sup>3</sup> This seems to indicate that a hydroxyl group  $\beta$  to the nitrosamine is a strong carcinogenicity reducing functionality.



**Figure 3. Structure of *N*-nitroso-diethanolamine (NDELA)**

**Group 1 (*N*-nitrosamines of  $\beta$ -blockers (Group 1a) and  $\beta$ -agonists (Group 1b) with *N*-isopropyl (or longer) groups)**

This group consists of the nitrosamines of the  $\beta$ -blockers acebutolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, pindolol, propranolol, sotalol, formoterol, and labetalol, as well as the  $\beta$ -agonists isoetharine, isoprenaline, metaproterenol (orciprenaline), sotalol, and formoterol.

Cross and Ponting (2021)<sup>4</sup> already identified that nitrosamines substituted by an isopropyl group (or longer) at one of the  $\alpha$ -carbon positions reduces the prevalence of carcinogenicity. They explain that “Substitution affects steric access to the  $\alpha$ -carbon. As steric bulk, such as isopropyl groups are added as part of the nitroso substituents,  $\alpha$ -carbon hydroxylation can become partially or totally inhibited due to steric hinderance. Consequently, not all nitrosamines will have the same unfettered ability to undergo  $\alpha$ -carbon hydroxylation as NDMA or NDEA.”

Thomas et al. (2022)<sup>5</sup> points out that the isopropyl group at the  $\alpha$ -carbon position is one of the two features associated with a large decrease in carcinogenic potency. They explain that “the presence of even one isopropyl group leading to a reduction in potency may be an extension of the observation that a *tert*-butyl group leads to an elimination of the potency and the reasons for it – while less sterically-hindered than a *tert*-butyl, the isopropyl is less likely to be a site of metabolism than a CH<sub>2</sub> group and, should metabolism occur on the other side of the nitrosamine, the formed diazonium or cation will be less reactive with DNA than a CH<sub>2</sub> group.”

Ponting et al. (2022)<sup>1</sup> also related to the effect that branched alkyl chains have on the carcinogenicity potency and they say that the introduction of steric hindrance at the carbon  $\alpha$  to the *N*-nitrosamine moiety has a dramatic effect on carcinogenic potency in animals. Branching in the form of a single methyl (or larger alkyl) group adjacent to the *N*-nitrosamine motif significantly reduces carcinogenicity and also the likelihood of genotoxicity. The presence of two such groups results in *N*-nitrosamines with minimal carcinogenic properties and mostly negative genotoxicity results. A potential reason for these observations is that the steric hindrance posed by the isopropyl-like  $\alpha$  substituent (even a mere methyl) perturbs  $\alpha$ -carbon hydrogen abstraction in the active site of CYP2E1 or CYP2A6 considerably, particularly for low-molecular-weight *N*-nitrosamines. Moreover, if one side of the *N*-nitrosamine molecule were to contain a methylene group and the other an isopropyl group, metabolic activation at the methylene group would lead to the formation of an isopropyl diazonium ion. Nucleophilic (SN2) displacement at this site by a nucleophile such as a DNA base is known to be a disfavored pathway. SN1 nucleophilic displacement (via the intermediate carbonium ion) at this site by a nucleophile such as a DNA base is likely to be disfavored as such a carbonium ion will likely react with solvent molecules (water) which are significantly more abundant.

These recent publications are in unison with respect to the potency lowering effect that an isopropyl substitution has on the carcinogenicity of nitrosamines.

### Comparison of induction time

Druckrey et al. (1967) reported on the carcinogenicity of various alkyl nitrosamines in BD rats.<sup>6</sup> When comparing doses that elicited tumors after the oral route of administration, as well as the average induction time, it is interesting to see that ethyl-isopropyl nitrosamine, as an exemplar of a nitrosamine with an isopropyl group at the  $\alpha$  position, has a longer induction time (375 days in liver and 345 days in esophagus), when compared to *N*-nitrosopiperidine (NPIP) that has two  $\text{CH}_2$  groups at the  $\alpha$  positions and has an established acceptable intake of 1300 ng/day (208 days in esophagus), even though the doses of ethyl-isopropyl nitrosamine were 2-4 times higher than the dose of NPIP (**Table 3**). This can be rationalized by considering that the steric hindrance of the isopropyl group disfavors the metabolic hydroxylation of the  $\alpha$ -carbon that is an isopropyl, and the  $\beta$ -hydroxy on the other side of the nitrosamine disfavors the CYP-mediated  $\alpha$ -carbon oxidation (*vide supra*). Together, this gives an overall reduction of carcinogenic potency, even when comparing to a weakly potent nitrosamine like NPIP.

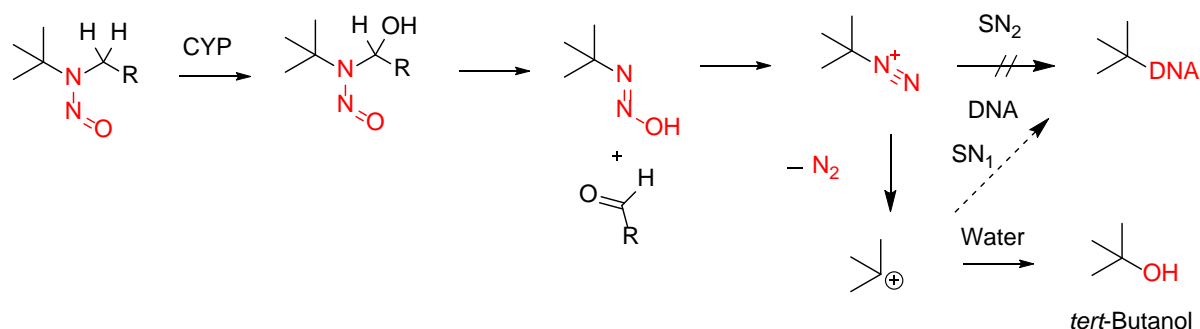
### **Group 2 (*N*-nitrosamines of $\beta$ -blockers (Group 2a) and $\beta$ -agonists (Group 2b) with *tert*-butyl groups)**

#### *Evaluation of Structure*

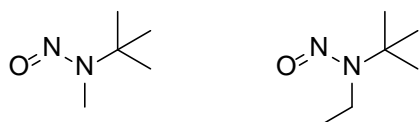
The nitrosamines with *tert*-butyl groups  $\alpha$  to the nitrosamine lack  $\alpha$ -carbon hydrogen atom on that side of the nitrosamine; therefore, the only diazonium ion that can form following  $\alpha$ -hydroxylation is *tert*-butyl diazonium ion (**Figure 4**). *tert*-Butyl diazonium ion is more hindered than isopropyl and significantly more than methyl or ethyl diazonium ions. In fact, the presence of a *tert*-butyl group on one of the carbons  $\alpha$  to the nitrosamine, has been shown to negate genotoxicity even when there is a metabolically labile methyl or ethyl group on the other side.<sup>4</sup> It is commonly accepted that nucleophilic reactions at a quaternary carbon via the  $\text{S}_{\text{N}}2$  mechanism is not favorable. Consequently, covalent adduction to DNA can only occur via an  $\text{S}_{\text{N}}1$  mechanism with the corresponding *tert*-butyl carbocation (**Figure 4**). However, such compounds have been reported to be non-carcinogenic.<sup>67</sup> The *tert*-butyl carbocation is likely to be preferentially quenched with water to form *tert*-butanol as an innocuous metabolite.

#### *Evaluation of Carcinogenicity Data*

Carcinogenicity studies were not identified in the Lhasa carcinogenicity database (LCDB)<sup>8</sup> for *tert*-butyl nitrosamines or even less hindered isopropyl nitrosamines. However, both methyl-*tert*-butyl nitrosamine (NMTBA) and ethyl-*tert*-butyl nitrosamine (EBNA) (**Figure 5**) have been reported as non-carcinogenic in the literature.



**Figure 4. Possible outcome of  $\alpha$ -hydroxylation**




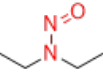

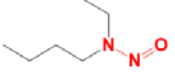
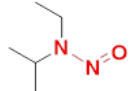
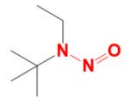
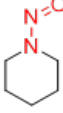
**Figure 5. Structures of NMTBA and EBNA**

#### Summary of Carcinogenicity Data

Gold et al. (1981)<sup>7</sup> reported that NMTBA did not induce tumors after subcutaneous injection of Syrian golden hamsters (15/sex/group) with doses of 0, 40, 80 or 160 mg/kg/week. It is possible that reduced survival (36-57 weeks in treated animals compared to 60-64 weeks in controls) impacted the outcome of the study. However, it can be concluded that NMTBA is not likely a potent carcinogen as other nitrosamines have been reported to induce tumors locally after subcutaneous injection in Syrian golden hamsters. For example, Gold et al. (1981)<sup>7</sup> notes that 1-acetoxypipylpropyl nitrosamine induced 90% incidence of sarcomas at the injection site with a short latency period (average 30 weeks), while NMTBA and acetoxymethyl-*tert*-butylnitrosamine, an activated form of NMTBA did not induce tumors.

Druckrey et al. (1967)<sup>6</sup> reported that treatment of 25 BD rats with 80 mg/kg/day EBNA in the drinking water to a total dose of 24 g/kg/day (i.e., 300 days of treatment) resulted in no tumors after an observation period of up to 730 days. Although carcinogenicity cannot be excluded due to the limited study design, EBNA is not a potent carcinogen when compared to other nitrosamines tested by Druckrey in similarly limited study design. When comparing doses that elicited tumors after the oral route of administration, as well as the average induction time, for other dialkyl nitrosamines to that for EBNA, which did not induce any tumors (**Table 3**), it is apparent that if EBNA is carcinogenic, it is not potent. More potent nitrosamines induced tumors at much lower cumulative doses and generally after a shorter period of time. For example, the potent carcinogen, NDEA, induced tumors in rats after daily oral treatment with a dose of 14 mg/kg/day for a total dose of 0.97 g/kg after an average induction time of only 68 days. Since the majority of nitrosamines tested by Druckrey do not have reported  $\text{TD}_{50}$  values or established acceptable intakes, the data for *N*-nitrosopiperidine was also compared, as this nitrosamine is not potent and has an established acceptable intake of 1300 ng/day.<sup>3</sup> Notably, Druckrey et al. observed tumors with an average induction time of 280 days and a total dose of *N*-nitrosopiperidine that is 17 times lower than that for EBNA, which did not induce any tumors after 300 days of treatment and 730 days of observation.

**Table 3. Comparison of oral carcinogenicity tumor induction dose and time for various alkyl nitrosamines tested by Druckrey et al. (1967)<sup>6</sup> in BD rats**

Nitrosamine	Daily dose (mg/kg)	Total dose (g/kg)	Average induction time (days)	Main tumor type	Established AI (ng/day)
Dimethylnitrosamine (NDMA) 	4	0.4	270	Liver, ethmoturb	96.0 <sup>9,10</sup>
Diethylnitrosamine (NDEA) 	0.075 to 14	0.064 to 0.97	840 to 68	Liver, esophagus	26.5 <sup>9,10</sup>
Methyl-ethylnitrosamine (NMEA) 	1 2	0.42 0.75	500 360	Liver	NA
Ethyl-n-butyl nitrosamine 	5 10	1 1.6	240 200	Esophagus	NA
Ethyl-isopropylnitrosamine (EIPNA) 	10 20	3.7 6.7	375 345	Liver, esophagus	26.5
Ethyl-tert-butyl nitrosamine (EBNA) 	80	24	(730)	No tumors	NA
N-Nitrosopiperidine (NPIP) 	5	1.4	208	Esophagus	1300 <sup>3</sup>

AI = Acceptable intake



The Group 2 nitrosamines have the same *tert*-butyl-nitrosamine scaffold as NMTBA and EBNA but with further substituents that are considerably larger and bulkier than the methyl and ethyl groups in NMTBA and EBNA. Accordingly, the activity of nitrosamines of  $\beta$ -blockers with *tert*-butyl groups at the  $\alpha$ -position can be read across from the published negative carcinogenicity data for NMTBA and EBNA and they can all be considered non-carcinogenic.

### **Group 3 (*N*-nitrosamines $\beta$ -blockers (Group 3a) and $\beta$ -agonists (Group 3b) that have two CH<sub>2</sub> groups at the $\alpha$ -position to the amine)**

This group consists of nitrosamines of two  $\beta$ -blockers, carvedilol and nebivolol and one  $\beta$ -agonists, vilanterol.

The group 3a compounds have CH<sub>2</sub> groups at both  $\alpha$ -positions to the amine, however, both  $\beta$ -positions have hydroxy or phenoxy groups. Following these hydroxy or phenoxy groups there are bulky cyclic substituents that add considerable structural hindrance to these two  $\beta$ -blockers.

Nitroso-vilanterol (Group 3b) also has CH<sub>2</sub> groups at both  $\alpha$ -positions to the amine, where one of the  $\beta$ -positions has a hydroxy group and the other has a long alkanoxy group terminated with a bulky cyclic substituent.

Furthermore, carvedilol is metabolized primarily by aromatic ring oxidation (hydroxylation via CYP 2D6) and glucuronidation;<sup>11</sup> nebivolol is metabolized mainly via direct glucuronidation and secondarily through CYP 2D6;<sup>12</sup> and vilanterol is principally metabolized by CYP 3A4 to a range of metabolites where the major route of metabolism was via O-dealkylation, and N-dealkylation and C-dealkylation were minor pathways.<sup>13</sup> The metabolic pathway leading to the  $\beta$ -carbonyl derivatives is not reported for any of these drugs, therefore, the  $\beta$ -hydroxy groups are considered carcinogenicity potency-reducing elements in the nitrosamine derivatives of carvedilol and nebivolol.

Combining the similarity of carvedilol and nebivolol to NDELA that has an established AI of 1900 ng/day,<sup>3</sup> with the added structural hindrance that these three compounds have, and with the competing metabolic pathways that degrade these  $\beta$ -blockers, it can be concluded that they are weak mutagens/carcinogens.

### ***In vitro* test results**

Several nitrosamines of  $\beta$ -blockers and  $\beta$ -agonists have been tested in the Ames test by various companies and the data was shared (anonymously) with the Lhasa Complex Nitrosamines Data Sharing Initiative (Vitic Complex Nitrosamines Database version 2022.2.0). The detailed data of these studies is restricted to the members of the data sharing initiative, however, from the 8 nitroso- $\beta$ -blockers currently listed in the Vitic database, four are negative (all from Group 1), three are positive (two from Group 1 and one from Group 3), and one is equivocal (from Group 1). To date no Ames test results for Group 2 nitroso- $\beta$ -blockers have been reported in the Vitic

database. Additionally, two nitroso- $\beta$ -agonists were tested and found to be negative in the Ames test. All the results were obtained in studies using a robust protocol with 5 tester strains, without and with metabolic activation using rat S9 (10%) and hamster S9 (10%), and using a standard preincubation procedure. The positive and equivocal results were all found in strain 1535 and only when hamster S9 was used for bioactivation. Preliminary bacterial mutagenicity data (generated by one EFPIA company) on two of these NDSRI's indicated that in certain cases a positive response in the presence of rat S9 can also be observed.

One nitroso- $\beta$ -blocker from Group 1 was tested in a HPRT gene mutation assay in Chinese hamster ovary (CHO) cells, with and without metabolic activation using rat S9. The results from this study indicated that the nitroso- $\beta$ -blocker was not mutagenic.

Further studies are required to determine whether the reported *in vitro* mutagenicity of these NDSRI's is indicative of subsequent *in vivo* mutagenicity.

### **Comparison with small dialkyl nitrosamines**

For nitrosamines that do not have robust carcinogenicity data available the current regulatory guidances advise to use a SAR approach to read-across from a structurally similar nitrosamine to derive a permissible AI. <sup>3,9,10,14,15</sup> The use of a SAR approach to set AIs for newly found nitrosamines, including nitrosamine drug substance related impurities (NDSRI) must be scientifically justified and properly documented.

NDSRIs are typically in a different chemical space than the simple alkyl nitrosamines (that form the basis of the current EMA NDSRI limits of 18 and 178 ng/day<sup>3</sup>) which are reported to be highly potent rodent carcinogens and as a consequence global structural similarity “read-across” approaches to assign a specific AIs for NDSRIs can be problematic. The carcinogenicity of nitrosamines in rodents is known to range across several orders of magnitude of potency,<sup>5</sup> and all highly potent nitrosamines that have robust carcinogenicity data curated in the LCDB<sup>8</sup>, are small molecular weight dialkyl nitrosamines. Therefore, it is not scientifically justified to use the established AIs of potent nitrosamines as surrogates for NDSRIs, and particularly for nitrosamines of  $\beta$ -blockers that are structurally much more complex and sterically hindered.

### **SUMMARY AND CONCLUSIONS**

All nitrosamines of  $\beta$ -blockers and  $\beta$ -agonists contain structural elements that impose considerable steric hindrance around the nitrosamine functionality. Most  $\beta$ -blockers and  $\beta$ -agonists have isopropyl or *tert*-butyl groups at the  $\alpha$ -carbon position, and the two  $\beta$ -blockers that have CH<sub>2</sub> groups at both  $\alpha$ -positions (carvedilol and nebivolol) are structurally similar to the weak carcinogenic NDELA and furthermore are substituted on both sides with bulky cyclic side chains. All  $\beta$ -blockers and  $\beta$ -agonists have  $\beta$ -hydroxy substituents next to the nitrosamine which are a further carcinogenicity potency reducing element, as these groups lead to increased polarity

and potentially disfavor CYP-mediated  $\alpha$ -carbon oxidation. The potency reducing characteristic of the  $\beta$ -hydroxy group is evident from NDELA that has two such substituents and is a weak carcinogen with an established AI of 1900 ng/day.

Ames test results for nitroso- $\beta$ -blockers are inconsistent, with some being clearly negative while others are positive in strain 1535 using hamster S9 (10%) and or rat S9 (10%) for bioactivation. HPRT of one nitroso- $\beta$ -blocker has resulted in negative mutagenicity. Further studies are required to determine whether the reported *in vitro* mutagenicity of these NDSRI's is indicative of subsequent *in vivo* mutagenicity.

In conclusion, a weight of evidence approach, together with the understanding that NDSRIs such as nitrosamines of  $\beta$ -blockers are structurally very different than the small potent nitrosamines that are currently being used as the default surrogates for setting AIs for nitrosamines, leads to the conclusion that nitrosamines of  $\beta$ -blockers are much less potent mutagens and their carcinogenicity potency is probably also much lower than the small nitrosamines. Until further data become available (e.g. from *in vivo* mutagenicity), it is proposed in the interim that nitrosamines of  $\beta$ -blockers be controlled as non-cohort-of-concern mutagenic impurities at the TTC limit of 1500 ng/day.

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## Acknowledgments

The authors thank Olivier Dirat (Pfizer), Jim Harvey (GSK), Susanne Glowienke (Novartis AG), Joerg Schlingemann (Merck KGaA), Ron Ogilvie (Pfizer), Andrew Teasdale (AstraZeneca), and Alison Dawson (Lilly) for their considered input and advice in the preparation of this paper.

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