CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20845

PHARMACOLOGY REVIEW(S)

Z. McDONALD

OCT 1 0 1997

REVIEW AND EVALUATION OF PRECLINICAL PHARMACOLOGY AND TOXICOLOGY DATA

NITRIC OXIDE

Ohmeda Pharmaceutical Products Division Inc
110 Allen Road
Liberty Corner, NJ 07938-0804

Narendra B. Oza, PH. D.
October 9, 1997

TABLE OF CONTENTS

OCT 1 0 1997

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Narendra B. Oza, PH. D.
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ORIGINAL NDA:	20,845
CENTER RECEIPT DATE:	June 17, 1997
REVIEWER RECEIPT DATE:	July 1, 1997
SPONSOR:	Ohmeda Inc.,Pharmaceutical Products Div. 110 Allen Road, PO Box 804, Liberty Corner, NJ 07938 (908) 604 7722
DRUG PROPRIETARY NAME:	I-NOTM (Nitric Oxide)
GENERIC NAME/CODE NAME:	N / A
STRUCTURE / NATURE:	N=O / Evanescent gas
MOLECULAR WEIGHT:	30
FORMULATION:	Formulated in 100, 400 and 1600 ppm nitric oxide with nitrogen (N_2) as the balance
RELEVANT IND'S:	NIH IND for respiratory hypoxia
PHARMACOLOGICAL CLASS:	Selective Pulmonary Vasodilator
INDICATION:	Respiratory Hypoxia of the newborn
DOSAGE:	5-80 ppm for up to 14 days
MODE OF ADMINISTRATION:	Inhalation

Pharmacodynamics:

A. Mechanism of Action:

NO is an endogenous, potent vasodilator substance. After its production , e.g., in vascular endothelial cells, the NO diffuses into smooth muscle cells, binds with the heme moiety of soluble guanylate cyclase and thus activates the enzyme. This enzyme increases intracellular cyclic guanosine 3',5'-monophosphate (cGMP) from its precursor, guanosine triphosphate (cGTP). The resulting increase in intracellular cGMP concentration leads to vasodilation.

B. Background:

NO, synonymous with EDRF (Endothelium Derived Relaxing Factor), is biosynthesized from L-Arginine by NO-Synthase primarily in endothelial cells. It is now becoming apparent that macrophages, neurons and many other cell types may also have a capability for local production of NO.

NO is an evanescent compound with half life of 41 seconds. NO is known not to be transported in the vascular bed and thus actions and interactions of NO are short term and local. NO has great affinity for hemoglobin. Following a very rapid binding with NO, haemoglobin is converted into methemoglobin (MHB) and the NO is inactivated. MHB is an abnormal, nonfunctioning ferric hemoglobin that is incapable of binding oxygen or carbon dioxide. Since MHB is unable to bind or release oxygen, any increase in its concentration can lower oxygen saturation in the blood and thus can become fatal. MHB level of 70% is usually fatal although a survival has been reported even after 81% level of MHB. The major enzyme responsible for the reduction of MHB is the NADPH dependent MHB reductase which is present in the erythrocytes. Spontaneous reduction of MHB is generally slow and methylene blue can serve as a cofactor by donating an electron and thereby increasing the amount of available NADPH. In vivo increase of NADPH can greatly accelerate the reduction of MHB.

The sponsors have proposed to monitor MHB levels and to terminate the inhalation of NO if the MHB level should exceed 5%.

C. Animal Studies on Inhaled NO:

1. Acute cardiovascular evaluation of NO in the dog via inhalation. Hassler CR et al, 1994 (Ohmeda PPD Report RDR-0064-DS;Battelle Labs Study Report #SC940065).
Location of Data: Vol. 2.8, p. 634 (final report) and Vol. 2.5, p. 42 (summary)

The purpose of this study was to evaluate in anesthetized laboratory beagles the physiological, pharmacological and toxicological effects resulting from acute inhalation of nitric oxide (INO). *Methods:* In comparison with control dogs exposed to 21% O_2 in N_2, the effects of 6 hr inhalation of 80 - 640 ppm NO were evaluated in groups of anesthetized, instrumented (inclusive of cardiac catheterization) dogs. In addition, animals were studied at 80 ppm using the same exposure device intended for clinical use. Continuous data were collected during exposures for systemic arterial and left ventricular pressures, minute volume, tidal volume, respiratory rate, pulmonary resistance, pulmonary compliance and pulmonary arterial pressure. Hourly determinations were made for cardiac output, ECG, MHB concentration and arterial blood gases. The concentration of NO and NO_2 were measured at the animal and the levels were as shown in the following Table 1.

Table 1: Protocol design of the Test Groups

Group No.	PPM NO±SD	PPM NO_2±SD	No. Of animals	Kg. Wt.±SEM
1	0	0	3	13.9 ± 1.0
2	81 ± 0.8	0.9 ± 0.2	4	14.7 ± 0.8
3 with clin. Dev.	80.4 ±1.5	1.0 ± 0.2	4	13.2 ± 0.4
4	160.7 ± 1.1	1.6 ± 0.3	3	14.2 ± 0.9
5	322.3 ± 1.2	5.6 ± 0.2	3	14.5 ± 0.4
6	640.6 ± 1.1	23.5 ± 0.9	3	13.3 ± 1.3

Results: The first physiologic response to NO exposure was dose-dependent, hourly increase in MHB concentration as shown in Table 2. This increase was concentration and duration related and statistically significant, $p < 0.05$, at 320 and 640 ppm.

Table 2
Group average percent Methemoglobin

Group	Baseline	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control	0.7	1.0	0.7	1.2	1.2	0.7	1.2
80 ppm	0.9	1.2	2.3	2.1	3.0	3.1	3.0
80 ppm with clin. Device	1.1	1.8	2.2	2.2	2.8	2.7	2.9
160 ppm	1.0	3.2	3.9	5.5	5.5	6.9	6.6
320	1.4	6.1*	10.5*	15*	18.1*	21.2*	24.1*
640	1.0	21.2*	36.3*	50.8*	65.1*	66.4*	78.1*

* $p < 0.05$

One of the three dogs treated with 640 ppm NO died with electrocardiographic changes. The ECG changes, found in all the NO-treated animals, consisted of Left ventricular depolarization (2 dogs 80 ppm and 1 at 160 ppm), Sinus tachycardia (3 animals), Junctional rhythm (2 animals at 80 and 160 ppm) and R on T phenomena (1 animal) at 640 ppm. The data suggested ventricular irritability and sinus tachycardia in dogs exposed to NO.

Arterial oxygen tensions (P_aO_2) declined throughout the exposure period and were significantly different from controls after 2 hrs exposure in the 320 and 640 ppm groups. Minute volume increased as a function of increased respiratory rate in the 320 & 640 ppm groups in an apparent response to the decreased oxygen tension. Systemic pressures decreased late in the experiment in the 320 & 640 ppm groups. A trend towards increasing heart rates was observed in <u>all</u> the dose groups, including 80 & 160 ppm.

<u>*Conclusions:*</u> Occurrence of premature ventricular depolarization (PVD's) lead to further investigate the potential of inhaled nitric oxide to cause the PVDs. The earliest response amongst many physiological changes was in the test-drug-dependant production of MHB; a concern which is prominent throughout this submission.

2. Electrocardiographic evaluation of NO in the dog via inhalation, Hassler CR et al, 1994 (Ohmeda PPD Report RDR-0087-DS; Battelle Labs Study Report #NO51491A).
Location of data: Volume 2.9, p. 804 (report) and Volume 2.5, p. 49 (summary)

This was a follow-up study to determine if INO produces abnormalities of cardiac conduction in normal unanesthetized dogs and to re-determine if the previously observed PVDs were incidental to anesthesia and cardiac catheterization.
Methods: Six beagles, 3/sex, were surgically implanted with ECG radiotelemetry transmitters and tracheal fistulae such that the conscious animals could be administered air or test drug during spontaneous breathing while sling restrained them for up to five hours. ECG data were continuously collected during the treatment which lasted five hours . Following a baseline obtained with ambient air, the animals were treated for the first hour with a controlled air mixture of 21% O_2 and 79 % N_2. Blood hemoglobin and MHB were measured hourly. Clinical observations and body weights were recorded regularly. All animals received nine treatments, 4 hrs each, which included: ambient air baseline, air control, and treatments with INO at 40-320 ppm. In one of the treatments (No. 2) a malfunction was found in the NO delivery system which resulted in a higher delivery of NO to test animals. Estimates of INO delivery were, therefore, calculated for treatment no. 2. The last 320 ppm treatment was preceded and followed by controlled air treatment. Two animals were treated per day with at least one week between treatments.

Results: With one exception (this animal had a prior abnormality of Wolf-Parkinson-White syndrome), INO up to 320 ppm did not affect cardiac conduction, rate or rhythm in normal, conscious, spontaneously breathing dogs including the treatment group no.2 in which instrument malfunction was noticed. The PVDs observed in the previous study were not found under these experimental conditions. The sponsors concluded that INO was unlikely to cause cardiac abnormalities.

Regarding methemoglobinemia, the sponsor noted that: "the blood MHB concentrations increased with increasing INO-exposures above 80 ppm with magnitude of the increases similar to those seen in the previous study". In fact, further calculation of the data reveal that animals exposed to 40 ppm NO also appear to increase MHB concentration. Although the increase in MHB caused by 40 and higher ppm of NO does not appear to cause apparent lung damage (see study no. 7), there are proponents that any level of MHB may be undesirable. The sponsor has defined severe methemoglobinemia at 7-10% MHB. In neonates 5% MHB was considered clinically significant. It appears that there may be a fair degree of tolerance of MHB due to improvements in perfusion and recovery of collapsed lung tissue. Furthermore, methemoglobinemia can be reversed by termination of INO and/or by iv infusion of vitamin C or methylene blue. Despite these favorable options, it appears prudent to abide by the recommendation of the sponsor to monitor MHB constantly and terminate INO when MHB level reaches 5%, particularly in view of the fact that the treatment is intended for PPHN.

Comment: Excepting the facts that the 320 ppm dose was cushioned with pre and post inhalation of air and that only two animals were used per dose, the data is agreeable with a notion that the physiological abnormalities observed in the previous experiment were related to anesthesia and catheterization. Blood MHB concentration of dogs treated with 40 ppm INO was not different from the controls suggesting a reasonable safety of low dosages.

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Pharmacokinetics:

3. Pharmacokinetic modeling of methemoglobin concentration--time data in normal dogs inhaling 80, 160, 320 or 640 ppm nitric oxide. Wilhelm JA, 1996; (Ohmeda PPD Report RDR-0075-DD)

Location of Data: Vol. 2.8, p. 756 (report) and Vol. 2.5, p. 264 (summary)

The purpose of this follow-up (see cardiovascular evaluation in # 1) study was to describe pharmacokinetics of inhaled nitric oxide in dogs. The animals of this study are exactly the same as those reported in review #1.

Methods: The data listed in table 2 (of review #1) were utilized to derive a model :

$$C = \frac{Vm\, C_{NO}}{K_m + C_{NO}} C_{NO}\,(1 - e^{-ke^{*}t}) + C_{base}$$

where C = percent MHB, C_{NO} = NO exposure concentration, K_e = MHB elimination rate constant, V_m & K_m = rate of MHB formation and C_{base} = baseline MHB level.

Results: Using this model and the kinetic data of table 2, the following parameters were estimated:

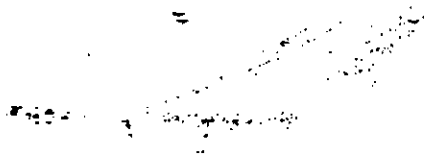
K_e (min^{-1})	Half-life (min)	C_{base}	V_m	K_m
0.0038	182	1.0	0.704	1992

Model-predicted, steady state percentage of MHB (C_{ss}) and MHB formation rates were:

Ppm NO exposure	Predicted C_{ss} (%MHB)	Rate of MHB increase (%/min)
80	3.5	0.0129
160	10	0.0345
320	32	0.1213
640	97	0.4297

Based upon the estimated elimination half-life of approx. 3 hrs, the time to reach steady state %MHB was approximated at 12 - 15 hrs. In a similar study performed in human volunteers, elimination half-life was approximated at 1 hr and the time to reach steady state was approximated at 4-5 hrs.

Conclusion: These data are obtained from the plasma samples of study #1 which was "discarded" because of artefact arising from anesthesia and catheterization. Thus the kinetic data, as well, can not be conveying anything meaningful.



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Toxicology:

4. 7-day range-finding study of nitric oxide in the rat via inhalation. Hassler CR et al, 1994. (Ohmeda PPD Report RDR-0062-DS; Battelle Labs Study Report #SC940063).

Location of Data: Volume 2.6, p. 283 (report); Volume 2.5, p. 139 (summary)

Study No./ Date: Report No. RDR-0087-DS; November, 1994

GLP Compliance: Yes

Animals: M/F Sprague-Dawley Rats/SD:CDBR

Administration: Inhalation

Dosage: 0 (20% O_2 + 80% N_2), 80, 200, 300, 400 or 500 ppm NO + 21% O_2 + Balance of N_2

Groups: Rats were exposed for 6 hrs/day at approximately the same time each day for periods of 1, 3, or 7 consecutive days at 5 concentrations of NO and air control (3 time points, 2 sexes, 5 rats/sex/time point and 6 treatments) as follows:

Dose	Animals M	Animals F	Treatment Days
Air control Gr.1	5	5	1
	5	5	3
	5	5	7
80 ppm Gr.2	5	5	1
	5	5	3
	5	5	7
200 ppm Gr.3	5	5	1
	5	5	3
	5	5	7
300 ppm Gr.4	5	5	1
	5	5	3
	5	5	7

400 ppm Gr.5	5	5	1
	5	5	3
	5	5	7
500 ppm Gr. 6	5	5	1
	5	5	3
	5	5	7

Observations:

Rats were observed for morbidity and mortality twice daily throughout the 7 days. Body weights were recorded once pre-study and on days 1, 3 and 7 of the exposure periods. A gross necropsy was performed on all surviving animals on days 2, 4 and 8 and after the last exposure of each dosing phase. A complete necropsy was performed on animals found dead or moribund. Histopathology was performed on tissues of the respiratory tract.

Results:

Mortality: There were no mortalities in air control, 80 ppm and 200 ppm groups. All the animals died on Day 1 in the groups treated with 400 and 500 ppm NO. Twenty six animals of the 300 ppm group died during days 1 & 2 and the four surviving were sacrificed on termination date. The survivors had cyanotic or bluish ears, nose and feet.

Clinical observations: Exposure-related discoloration (bluish tint) of the skin in the 300 ppm gr and red nasal discharge were noted in all the drug-treated animals.

Methemoglobinemia: Percentage of MHB concentration of all the groups are shown in the following table no. 3.

Table 3

Percent Methemoglobin:

Day	Cont.	80 ppm	200 ppm	300 ppm	400 ppm	500 ppm
1	2.7	2.7	22.0	67.0	72.5	57
3	1.6	1.6	24.6	na	na	na
7	2.3	3.7	22.7	38.1	na	na

From this data the sponsors concluded that "80 ppm animals were not distinguishable from the control". Although it appears that way, one can not ignore the high reading of 3.7 % MHB obtained with 80 ppm NO on day 7 (see table 3). Thus it seems that 80 ppm is alright for 3 days but not for 7 days. The NO-induced methemoglobinemia was associated with increases in blood urea nitrogen, potassium and aspartate amino transferase, particularly in animals treated at ppm of 300 & higher.

There were sporadic changes (decrease in total protein, increase in blood glucose, increase in creatinine etc) in some parameters which did not establish a trend and could not be related to treatment.

Light Microscopy: Several rats had a focal-multifocal, minimal inflammatory foci and/or microgranulomas in the lung parenchyma. Since these changes were found also in the controls, the sponsors concluded that these changes were random, spontaneous and unrelated to agent exposure.

Comment: The sponsor attributes most of the toxicity of NO to the formation of MHB. There are no fewer than a dozen different excitatory agents (histamine, prostaglandins, bradykinin, SRS etc) thought to act as humoral mediators for the onset or reversal of pulmonary inflammation. None of them are either measured or taken into account. Although the sponsor suggests that "dosages higher then 80 ppm promote methemoglobinemia in SD rats", the exclusion of 80 ppm is without rationale and is not very convincing.

5. 7-day range-finding study of nitric oxide (NO) in the rat via inhalation, Toft JT and Singer AW, December 1996 Final Report (Ohmeda PPD Report RDR-0151-DS; Supplemental Report to Battelle study #SC940063).
Location of data: Volume 2.9, p. 936 & Volume 2.5, p.149.

This is an Anatomic Pathology part of the toxicology study outlined in the preceding.

External carcass and internal organs were examined with a particular attention to the respiratory tract. The lungs were examined for petechiae, macroscopic foci of inflammation, edema and possible discoloration due to methemoglobinemia.

Abnormal morphology, oral and nasal passages and evidence of exudate, foam or edema were also recorded. *Results:* No insult was found in the epithelium of the upper respiratory tract which is more vulnerable to inhaled toxicants. Light microscopy did not reveal loss or damage to cilia and ciliated cells. No evidence of toxicity or alteration was found in the mast cells. There was also no evidence of toxicity in terminal bronchioles. *Comment:* It is somewhat surprising that the respiratory tract was unaffected despite a 100% mortality and a high degree of methemoglobinemia found at higher dosages. Is it possible that light microscopy is unable to detect these changes ? Fortunately an answer was found in the EM data which follows.

6. Electron microscopy study report: 7- day range-finding study of nitric oxide in the rat by inhalation, Mann P. et al, August 1996 Final Report (Ohmeda PPD Report RDR-0149-DS; Experimental Pathology Laboratories Final Report #541-001)

Location of Data: Volume 2.9, p. 877 (report) & Volume 2.5, p. 152 (summary).

This study examines the ultrastructural effects on rat bronchiolar and respiratory tissue samples obtained from the toxicology study (#SC940063) described above.

Lung specimens of the male rats (5 / group), shown in the following table 4, were examined by electron microscopy. In choosing these samples one wonders why the low dose, 80 ppm, and the fatal dosages were excluded. The selection of mid-dose, 200 ppm, is difficult to rationalize because the actual clinical use may be well under 80 -160 ppm.

Table 4

Ppm NO Targeted	Ppm NO achieved	Ppm NO_2	No. Of exposures	Group No.
0	0	0	1	1/1
200	198	2.2	1	1/3
0	0	0	7	7/1
200	202	2.2	7	7/3

The lung sections were examined at the Laboratory for advanced electron and light optical methods, College of Veterinary medicine, N. Carolina state university, Raleigh. A low power and a high power photomicrographs were taken on coded specimens and were decoded after the evaluation by a pathologist. *Results:* Intersticial edema has been reported as an early change produced by exposure to relatively low concentration of NO_2. In this study, the incidence of edema was greater relative to control but similar for both 1 and 7-day animals. The severity of edema was graded

"moderate" in animals sacrificed after 1 day and "slight/mild" in animals sacrificed after 7 days. This observation indicated signs of transient oxidative injury to the respiratory epithelia at 200 ppm NO and 2.2 ppm NO_2 in rats. *Conclusion:* Exposure of rats to 200 ppm NO and 2.2 ppm NO_2 promotes oxidative insult to respiratory epithelia. The sponsor graded it to "lowest-observable-effect level". Whether such an insult was apparent also in the low dose groups remains unknown.

7. 28-day exposure with recovery of nitric oxide in the rat by inhalation. Hassler CR et al. 1994 Final Report. (Ohmeda PPD Report RDR-0063-DS; Battelle Study Report #SC940064)

Location of Data:	Volume 2.7, p. 491 (report) &Volume 2.5, p. 166 (summary)
Study No./ Date:	Report No. RDR-0063-DS; November, 1994
GLP Compliance:	Yes
Animals:	M/F Sprague-Dawley Rats/SD:CDBR
Administration:	Inhalation
Dosage:	0 (air), 40, 80, 160, 200 and 250 ppm NO in 21% O_2 administered 6 hrs/day,7 days/week through nose only delivery system of inhalation. Nose only inhalation system allowed the administration of high concentration of NO without excessive oxidation to other oxides, in particular NO_2. This was accomplished by diluting the NO into a nitrogen atmosphere and adding oxygen immediately prior to administration. The test article concentrations were measured using chemiluminescent monitor which determines NO / NO_X (NO_X =total oxides of nitrogen). A solenoid valve was used to direct the sample flow through the catalytic convertor to measure NO_X or bypass the convertor to measure NO. The oxygen level was monitored using Airway Gas Monitor. Both instruments were calibrated before and during the experiment. Each level of the exposure group was monitored for NO, NO_X and O_2 in a continuously rotating sequence from the lowest to the highest dose groups. Each exposure level was monitored at least five times during the daily 6 hr exposures. The

effective concentration of inhalants was as described in table No. 5.

Table no. 5

Group	NO M±SD ppm	NO_x M±SD ppm	O_2 M±SD %	NO_2 M±SD ppm
1. Air Cont.	NA	NA	21. 3±0.9	NA
2. 40 ppm	40. 3±1.3	40. 5±1.4	21. 5±0.8	0.2±0.4
3. 80 ppm	79. 8±3.9	80. 4±3.9	21. 3±0.9	0.6±0.4
4. 160 ppm	159.5 ± 10	161.1 ± 10.1	21. 3±0.7	1.6 ± 0.6
5. 200 ppm	198 ± 6.4	200.6 ± 6.6	21.2 ± 1.1	2.6 ± 0.9
5. (a) 200 ppm	198.6 ± 2.2	200.9 ± 4.0	21.4 ± 0.9	2.3 ± 3.4
6. 250 ppm	247.0± 20.9	250.5 ± 21.2	21. 6±0.7	3.5 ± 0.9

Groups:

10 /sex/gr allocated for terminal sacrifice on Day 29
5 /sex gr allocated to 28 day recovery period.
Additional 8 M & 5 F were added to 200 ppm group because of instrument malfunction that occurred.

Observations:

Rats were exposed for 6 hrs/day, 7 days / week. The concentration of NO, NO_X and O_2 were measured as described above. Body wts., food consumption, clinical observation and clinical pathology determinations were made periodically. Gross necropsy was performed on all animals. Histopathology was performed on all animals of the control and 200 ppm groups.

Results:

Mortality: There were no deaths in 40, 80 & 160 ppm groups. One F in 200 ppm (gr. 5a), and 19 of the 30 in 250 ppm group died within the first two days of

exposure. An instrument malfunction (although this malfunction is stated here very casually, it reaffirms my doubts that a similar malfunction may be impossible to avoid) resulted in overdosing by 32% which, apparently, killed 6 animals in the 250 ppm and 10 in the 200 ppm groups. Another point of disturbance was in the fact that, if it was an instrument malfunction to overdose by 32%, I would have expected more deaths in the 250 ppm group rather than the 200 ppm group unless one interprets that NO doses of 200 ppm and higher can be randomly fatal. A group of 13 animals was added as a replacement for 200 ppm but not for the 250 ppm under an argument that mortalities were high at 250 ppm. In fact mortalities were higher in the 200 ppm group. Of the replacement, 1 F died on day 1 and 1 M died on day 15th of the exposures.

Rapid respiration, ataxia, lethargy and blue & pale skin texture were noteworthy clinical observations which, according to sponsor, were primarily limited to the 200 & 250 ppm exposure groups. Body weights and food consumption were unremarkable. Clinical pathology revealed dose-related elevations in methemoglobin; "the only toxicologically significant finding" as per the sponsors. It is noted that MHB levels were higher only when blood samples were taken immediately after the NO exposure. As expected, these levels had returned to normal 24 hrs after or after 28 days of continuous treatment.

Gross necropsy revealed a pale tan to brown coloration in the lungs of the animals treated with 200 & 250 ppm NO. Organ weights were unremarkable. Light microscopic evaluations did not reveal NO-related damage.

Methemoglobinemia: The most significant reason of death was methemoglobinemia (as shown in Table 6) resulting in histotoxic anoxia.

Table 6: NO induced Methemoglobinemia

Gr.	1	2	3	4	5	6
p p m NO	0	40	80	160	200	250
D a y 7	2.0	2.6	2.9	16.1	27.4	N o data
D a y 14	1.8	2.1	9.3	32.3	38.7	N o data
D a y 21	1.3	1.3	2.3	17.3	25.1	N o data
D a y 28	1.4	1.9	2.2	16.2	34.8	39.0
D a y 29	1.8	1.9	2.6	2.1	2.4	2.3
D a y 56	1.7	2.0	2.0	2.2	2.0	N o data

If one disregards the time factor (days 7-56) of determination, normalized average % MHB appears to increase with INO as calculated below :

Table 6a: Normalization of Methemoglobinemia

PPM	0 (air)	40	80	160	200	250
% MHB	100	118	213	860	1301	NA

The sponsors state that "the effects were not readily discernible at exposures of 80 ppm and lower (see Table 6) dosage". I think the trend of methemoglobinemia is apparent even at the smallest dose as shown in Table 6a. In comparison with air control, the average MHB appears 118% and 213% at 40 & 80 ppm respectively. In view of the high affinity of NO for Hb, I will be surprised If MHB was not formed. In the absence of fatality, at the best, this data indicates that rat is tolerant of methemoglobinemia invoked by 40 - 80 ppm INO.

Conclusion: Methemoglobinemia appears to occur even at the lowest tested dose. Although there were no fatalities amongst the low dose groups, concerns on MHB continue to persist.

8. 28-day exposure with recovery of nitric oxide in the rat via inhalation, Singer AW., December 1996, Final Report (Ohmeda PPD Report RDR-0152-DS)

Location of Data: Volume 2.10, p. 1004 (report) and Volume 2.5, p. 171 (summary).

This is an Anatomic Pathology part of the toxicology study outlined in the preceding.

External carcass and internal organs were examined with a particular attention to the respiratory tract. The lungs were examined for petechiae, macroscopic foci of inflammation, edema and possible discoloration due to methemoglobinemia. Abnormal morphology, oral and nasal passages and evidence of exudate, foam or edema were also recorded. *Results:* No insult was found in the epithelium of the upper respiratory tract which is more vulnerable to inhaled toxicants. Light microscopy did not reveal loss or damage to cilia and ciliated cells. No evidence of toxicity or alteration was found in the mast cells. There was also no evidence of toxicity in terminal bronchioles. *Comment:* It is comforting to note the absence of abnormal morphology in this study. However a possibility can not be excluded that light microscopy was unable to detect finer changes as in the previous study.

9. Electron microscopy study: 28-day exposure with recovery of nitric oxide in the rat by inhalation; Mann P. et al, August 1996 Final Report #541-002 (Ohmeda PPD Report RDR-0150-DS;)

Location of data: Volume 2.9, p.909 (report) & Volume 2.5, p.174 (summary)

Ultrastructural effects of NO exposure were investigated in bronchiolar, transitional and respiratory tissue samples of the animals of the foregoing toxicology study (no. RDR-0063-DS). *Methods:* Lung specimens from 5 M rats were processed from groups 1 & 5 (see table 6) exposed at 0 and 200 ppm NO respectively. It is unclear why the groups with low dosages, 40 & 80 ppm, were not examined. Tissue sections were prepared using standard processing techniques for electron microscopy. Four grids were examined from each lung lobe. Terminal bronchioles and adjacent alveoli were examined and photographed when both areas were present. All the photomicrographs of the control animals were examined first to establish background changes and then randomized with the treated animals, coded, and then given to a pathologist for evaluation. *Results:* The ultrastructural changes included spreading of type II cells, decreased number / size of cilia in ciliated respiratory epithelial cells, electron lucent areas in clara cells and altered shapes or decreased height of clara

cells. Since these changes were found in treated animals as well as in controls, the sponsor does not relate them with the drug treatment. *Conclusion:* Since only the low doses of INO may have a chance for therapeutic use, I would have liked to see the EM data of low dose groups just to reassure that the findings are similar.

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ON ORIGINAL

Review of pertinent literature:

The sponsors have cited a number of references from which a selected few, addressing the issues of efficacy and safety are reviewed. Since the division has asked the Pharmacology Reviewer to comment on *Tolerance and Withdrawal,* this topic is also included in the following review of literature:

Efficacy:

(i) Nitric Oxide reverses Acute Hypoxic Pulmonary Hypertension in the Newborn piglet: Etches et al, Pediatric Res., 35, 15-19, 1994.

The aim of this study was to investigate the feasibility of administering NO to 1-2 day old piglet (neonatal model), to evaluate the dose response characteristics and to determine the time course for effect of NO on hypoxic pulmonary vasoconstriction. Methods: Newborn piglets were instrumented to allow measurements of cardiac index, pulmonary arterial pressure and systemic arterial pressure. Pulmonary hypertension was induced by reducing the fraction of inspired oxygen to 0.12 -0.14. The animals were then given 5-80 ppm NO. Results: All concentrations of NO were associated with a rapid decrease in pulmonary arterial pressure and pulmonary vascular resistance. Cardiac index increased and systemic vascular resistance decreased, once again at *all dosages.* Very important finding was in the fact that there was no significant difference noted between the various doses of nitric oxide. Stated in other words, the pulmonary vasodilator response of 5 ppm was the same as that of 80 ppm NO. The investigators further stated, and I quote, that " although there appears to be a consistent maximal effect with 80 ppm in the newborn piglets, we found no statistical advantage in going beyond 5 ppm in terms of the absolute decrease in PAP and reduction of PVR". Thus, assuming that human newborns may be approximately twice the weight of piglets (approx. 1.8 kgm), there may not be a need to exceed 10 ppm NO; a maximum approvable dose. Conclusion: The main concern of NO-therapy is toxicity due to methemoglobinemia and pulmonary injury due to NO_2. Both of these issues are likely to be diluted out by the use of very low levels, eg 5 ppm, of NO. Furthermore, as stated in the following publication by Romand et al, all of the pulmonary vasoconstriction is not reversible by INO anyway, then why risk the safety by administering higher dosages of NO ?
Although there are other studies (eg, Rich et al, J. Appl. Physiol., 75: 1278-1284, 1993 which used isolated rat lung model) suggesting dose-dependant vasodilatory effects up to 1000 ppm NO, those studies have employed either isolated organs or have employed adult animals. The data generated in newborns animals are somewhat direct and provide a better guideline despite the absence of symptoms of PPHN. The preclinical animal studies, thus, indicate that whatever beneficial effect is likely to be derived from the NO therapy, is indeed offered by low doses INO. Until additional evidence is available on safety, it may be prudent to use the very low doses of INO.

(ii) Hemodynamic effects and metabolic fate of inhaled nitric oxide in hypoxic piglets: Jacob et al. 76: 1794-1801, 1994.

In this paper Jacob et al confirm above reviewed observations of Etches et al. The data demonstrated pulmonary vasodilatory properties of NO in piglets with hypoxia induced by hypoxic ventilation. The antihypertensive response of 5 ppm NO did not improve further with a higher dose of 40 ppm. Thus, the findings in piglets appear in contrast to other observations made in sheep and rat which suggest dose-dependant improvement of vasodilation. Despite this divergence of data, and unless proven otherwise (by animal models which truly replicate human infants), I would tend to hypothesize that the INO therapy in human infants may show all the total benefits at low dosages without further improvements at higher dosages (similar to that found in the present and the previous study of Etches et al) and recommend the use of INO therapy at / around 10 ppm. Of course, the review of the Medical Officer can supersede this recommendation if he/she has a more compelling, direct evidence.

(iii) Inhaled Nitric Oxide partially reverses Hypoxic Pulmonary vasoconstriction in the dog: Romand et al. J. Appl. Physiol., 76(3), 1350-1355, 1994.

The purpose of this study was to characterize interaction between NO and hypoxic pulmonary vasoconstriction (HPV) in a canine model. The authors hypothesized that NO inhalation would *completely* reverse the increase in pulmonary vasomotor tone during hypoxia and that the time course of this response may follow zero-order kinetics. Methods: Arterial and venous systemic and pulmonary pressures, flow-probe derived cardiac output, nitrosyl-haemoglobin (NO-Hb) and Methemoglobin (MHB) were determined in hypoxic pulmonary vasoconstricted, anesthetized and ventilated dogs. Results: Hypoxia induced an increase in Ppa that reached a plateau after 5 min. Exposure to 28 & 47 ppm NO induced an immediate (within <28 seconds) decrease in Ppa & Pvr *but did not return either to baseline hyperoxic values.* Increasing the concentration of NO to 74 and 145 ppm in two dogs during hypoxia did not induce any further decreases in Ppa. Reversing hypoxia while NO remaining at 47 ppm further decreased Ppa and Pvr to baseline values. NO inhalation did not induce decreases in systemic arterial pressure. MHB was either unchanged (0.6%) or increased to 1.1% and NO-Hb was not detectable. Conclusion: NO-inhalation caused only partial reversal of hypoxia-induced increase in pulmonary vasomotor tone in this canine model. The vasodilatory effects of NO were immediate and selective to the pulmonary circulation.

Safety:

Dysfunction & Toxicology:

(iv) Surfactant dysfunction after inhalation of nitric oxide: Hallman et al. J. Appl. Physiol., 80(6) , 2026-2034, 1996.

The objective of this study was to determine if INO has any detectable effects on the surface activity of bronchioalveolar lavage (BAL) return. It was also of interest to determine whether surfactant damage could result from a direct effect on pulmonary surfactant or from an effect on proteins that inactivate surfactant. Methods: Surfactant function was investigated in 36 M, young rats following humidified inhalation of either a) air b) 95% O_2 c) air and 100 ppm NO or d) 95% O_2 and 100 ppm NO. The treatments did not change the recovery of phospholipid from BAL. Exposure to NO of animals that breathed air or 95% O_2 increased the minimum surface tension (1.6 to 2.4 times) of surfactant from BAL at low, 1.5 µmol/ml, but not at high, 4 µmol/ml, phosphatidylcholine concentration. After inhaled NO, the nonsedimentable protein of BAL decreased the surface activity of surfactant (1 µmol/ml phosphatidylcholine/ml) more than the protein from the controls. NO treatment of animals that breathed either air or 95% O_2 affected neither the quantity nor the mol. wt. distribution of nonsedimentable protein. Hyperoxia increased the amount of the nonsedimentable protein, whereas NO increased the iron saturation of transferrin. The surfactant fraction and the nonsedimentable protein from BAL were separately exposed to 80 ppm NO in vitro. NO exposure had no effect on the surface activity of surfactant fraction. NO exposure of nonsedimentable protein from the control animals (no NO) increased the inhibition of surface activity and changed the adsorption spectrum of the protein, suggesting conversion of Hb to MHB. Nonsedimentable protein from NO-exposed animals contained MHB. Conclusion: The investigators proposed that surfactant dysfunction caused by inhaled NO is in part due to alteration of proteins in epithelial lining fluid that in turn inactivates surfactant.

(v) Lung Surfactant Components in Bronchiolar lavage after inhalation of NO_2 as markers of altered surfactant metabolism: Muller et al. Lung, 172 , 61-72, 1994.

This study was intended to evaluate dose related effects of in vivo exposure of NO_2 on surfactant components of rat lung. Methods: After exposure of 0.8, 5 and 10 ppm NO_2 for 1-3 days, lung lavage was analyzed for surfactant components as well as lavageable cells. Results: Following NO_2 exposure, there was an increase in lavageable cells with notable elevations in the number of granulocytes and lymphocytes but the macrophages had decreased. There also was an increase in total protein content of the lavage which was dose related. Phospholipid was increased with a decreased portion of phosphotidylcholine (PC). Further analysis of PC showed a decrease in total but an elevation in unsaturated fatty acids. Surface tension of

surfactants exposed to 0.8 ppm was not altered. It was increased 14% ($p<0.01$) and 27% ($p<0.01$) in the animals exposed to 5 and 10 ppm NO_2 respectively. Conclusion: The data clearly demonstrated that NO_2 inhalation impaired function of surfactant components which are used as markers of altered surfactant metabolism.

(vi) Inhibition of alveolar type II cell ATP and surfactant synthesis by nitric oxide; Haddad et al. Am. J. Physiol., 14, L898-L906, 1996.

Since alveolar type II (ATII) cells are often exposed to increased concentration of endogenous and exogenous NO, the effect of such an exposure was investigated in the synthesis of surfactants. Methods: Freshly isolated rat ATII cells were exposed for 2 hrs at 1-3 μM NO (generated by S-nitroso-N-penicillamine, spermine NONOate, or 3-morpholino -sydnonimine in the presence of duperoxide dismutase) which resulted in ~60% decrease in the rate of surfactant synthesis, as measured by the rate of incorporation of [methyl-^{3}H] choline into phosphatidylcholine and 60-80 % inhibition of cellular ATP levels as determined by bioluminescence. Results: Similar results were obtained after incubation of ATII cells with peroxinitrite but not sin-1, a putative generator of peroxinitrite. Addition of 20 μM oxyhemoglobin (scavenger of NO) or enhancement with glutathione ester totally prevented the NONOate inhibition of cellular ATP. In contrast to the in vitro findings, normal levels of ATP and lipid synthesis were measured in ATII cells isolated from the lungs of rats that breathed 80 ppm NO in 21% O_2 for 2 hrs. This lack of effect may be due to the presence of various antioxidants (like glutathione) in the epithelial lining fluid or to relatively low concentration of NO reaching the alveolar epithelium. Conclusion: The data suggested that NO and peroxynitrite, at concentrations likely to be encountered during in vivo inflammation, decrease ATII cell energy stores and surfactant synthesis, which may lead to derangement of important physiological functions. This decrease in surfactant level appears consistent with increased surface tension of BAL surfactant seen in previous studies.

(vii) Nitric Oxide and Nitrogen Dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods, Stavert and Lehnert, Inhalation Toxicology, 2:53-67, 1990

The purpose of this study was to examine the pulmonary inflammatory response to nitric oxide relative to that of Nitrogen Dioxide when breathed at high concentrations for short durations. The objectives were accomplished by using exposure system and exposure procedures that provided means to expose rats to high concentrations of NO while minimizing contamination due to NO_2. Methods: Adult male (Fischer) rats were exposed to 500-1500 ppm NO for 5-30 min using protocols which limited concentration of contaminating NO_2 to <30 ppm. Other groups were acutely exposed to 10-100 ppm NO_2 for 5-30 min. The control group was exposed to filtered air. Twenty four hrs after the exposure, the animals were sacrificed and the lungs were examined

by gravimetric and histopathologic analysis. Results: The wet weights of lung and right cranial lobe were not affected by exposure of rats up to 1500 ppm NO / 15 min or 1000 ppm NO /30 min . Likewise, such exposures did not induce any histopathological changes. There were no changes also in rats exposed to 10 or 25 ppm NO_2 /30 min or those exposed to 50 ppm NO_2 for 5 or 15 mins. The lung wts. were increased 24 hrs after a 30 min exposure to 50 ppm NO_2 and after 5-15 min exposure to 100 ppm NO_2. Histologic lung injuries were observed following 30 min exposure to 25 ppm NO_2 but not at lesser exposure or times. A 15 min exposure to 100 ppm NO_2 resulted in the most severe injury. Conclusion: These results suggested that shorter durations of even high concentration of NO did not cause lung injury. Histologic disturbances were evident 24 hrs after rats were exposed to 50 ppm NO_2 / 30 min.

(viii) Bleeding time and NO inhalation, Hogman et al, Lancet, 341: 1665 Letter, 1993

In this letter the authors reported prolongation in bleeding time after NO administration in rabbits and man. Rabbits were exposed for 30 and 300 ppm NO over 15 mins. The bleeding time increased 46 ± 14 % ($p<0.001$) during 30 ppm and 72 ± 20% ($p<0.05$) during 300 ppm NO inhalation. Subsequently, the effect of INO on the bleeding time was also determined in six health volunteers. After a standard incision on the ventral part of the forearm, the blood was blotted with a filter paper every 10 seconds until no blood appeared on the paper. The bleeding time was measured before, 15 min after inhaling 30 ppm NO and 30 min (n=3) and 60 min (n=3) after stopping inhalation of NO. The ratio of bleeding time increased to 1.33 ± 0.5 ($p<0.001$) after 15 min of inhalation of 30 ppm NO. Thirty min after the withdrawal of NO, the mean ratio had fallen to 1.14 ± 0.05 in 3 volunteers. The other three returned to pre-inhalation level, 1.04 ± 0.01 after 60 min. Conclusion: The authors did not have any clue behind this systemic effect and recommended to bear in mind that patients on INO may have increased risk of bleeding. How this disfunction can affect infants was not clear from this data.

(ix) Caution with use of inhaled nitric oxide, Warren & Higenbottam, Lancet, 348: 629 (commentary), 1996.

This communication outlines approved treatment for systemic vasodilation, summarizes pro and cons of NO therapy and recommends that INO should not be used as a routine until such time that certain requirements are met. Intravenous epoprostenol is an approved, short acting drug for pulmonary hypertension but it worsens pulmonary gas exchange in ARDS patients. Inhaled preparation can be an advantage in ARDS particularly if it is short acting in improving regional gas exchange without causing systemic hypotension. INO is a good candidate because it meets these requirements. NO is a corrosive gas stored diluted in nitrogen to avoid explosion. Clinically effective doses are 0.1 - 100 ppm. Higher concentrations cause pulmonary edema and methemoglobinemia. Much of the toxicity is due to NO_2 which

is 5-20 times more toxic than NO. The rate of oxidation of nitric oxide is slow but increases in high oxygen concentration, such that with 100% O_2 the toxic level of NO_2 is formed within 23 seconds. NO can be toxic because it is a free radical and because of its metabolic intermediates such as perioxynitrite. A large portion, almost 70-80%, of inhaled NO forms nitrate, the remainder is excreted as urea from an unknown metabolic pathway. Nitrite is also formed when NO dissolves in aqueous phase and is present in the plasma at 1.3 - 13 µmol / L. Nitrite, unlike NO, can nitrosate sulphahydryl groups on, for example, ion channels or circulating oxyhemoglobin. Also unlike NO, nitrite anions could contribute to neural toxicity and potential carcinogenicity of inhaled NO. There is an absence of regulatory approval (in U.K.) and there are no known specifications for gas cylinder connectors or a pin index system as for other medical gases to prevent wrong use of a cylinder. The possibility of accidental overdosing remains because there is no color coding of NO cylinders. *Conclusion:* These authors did not recommend routine use of INO in clinical practice and quoted that "UK committee on safety of Medicine has reminded doctors that they are (themselves) responsible for any adverse consequences of using an unlicensed medicine (INO)".

Tolerance:

The following is cited with a note that the information on these topic is rather limited.

(x) Inhaled Nitric Oxide: A Selective Pulmonary Vasodilator Reversing Hypoxic Pulmonary Vasoconstriction. Frostell et al. Circulation. 83. 2038-2047. 1991.

Background: The investigators examined the effects of inhalation of 5-80 ppm NO gas on the normal and acutely constricted pulmonary circulation in awake lambs.

Methods: In two experiments pertinent to *tolerance,* the pulmonary circulation of awake and spontaneously breathing lambs was acutely constricted with infusion of the thromboxane endoperoxide analogue, U46619.

Experiment 1: Eight lambs breathed a series of NO (5-80 ppm NO) and O_2 mixtures for 6 mins. Each level of NO exposure was followed by 6 min of breathing the oxygen mixture without NO. Then, a second exposure of 5, 10, 20 ppm NO for 6 min was performed and each lamb was examined for the occurrence of acute *tolerance.* Subsequently, each lamb was studied during a control period of breathing the oxygen mixture 6 min. after ceasing U46619 infusion. MHB levels were studied before and after the study.

Experiment 2: Four awake lambs were given U46619 infusion to raise their mean PAP to 30 mm Hg. After initial measurement of pulmonary and systemic hemodynamics, the lambs inhaled 80 ppm NO at FiO_2 of 0.6-0.7 for 1 hr. Repeated measurements were

obtained at 3, 6, 15, 30 and 60 mins of NO breathing and again at 3 and 6 min after ceasing NO breathing. Then, U46619 infusion was discontinued and haemodynamics were determined after 10 mins.

Results:

Expt. 1: NO-inhalation reduced pulmonary hypertension in seconds and the vasodilator effect was maximal in 3 min. Termination of NO-inhalation caused a return to the previous level of vasoconstriction. The vasodilator response of the second exposure was the same as the first exposure.

Expt. 2: Inhalation of 80 ppm NO produced sustained pulmonary vasodilation to a normal PAP and PVR for 1 hr. The pulmonary hypertension promptly recurred after cessation of INO. There was no short-term *tolerance* to vasodilation. MHB levels were 1.1 ± 0.25 % after 1 hr of 80 ppm INO which did not significantly differ from the control value of 0.68 ± 0.14 %.

Conclusion: *Tolerance* to nitric oxide was not evident in these experiments in terms either of the vasodilatory effect of NO (determined at 6 min apart !), *per se*, or the MHB levels (determined 1 hr apart) which did not differ significantly from the control.

Withdrawal:

(xi) Inhaled Nitric Oxide for the Adult Respiratory Distress Syndrome: Rossaint et al, New Engl. J. Med., 328 , 399-405, 1993. (Since this article is a clinical effort, it might be reviewed in detail by the Medical Officer Reviewer).

Rossaint et al investigated whether INO would cause selected vasodilation of ventilated lung regions, thereby reducing pulmonary hypertension and improving gas exchange. Seven patients were treated with 5-20 ppm NO for prolonged inhalation of 3 - 53 days. The administration of INO was discontinued daily for 30 mins with the ventilator settings kept constant and the FiO_2 set at 0.90-0.98 to determine the effect of *withdrawal* and resumption of treatment on hemodynamic effects. Treatment with NO was terminated after weaning from extracorporeal membrane oxygenation (ECMO) was successful ($PaO_2 / FiO_2 > 250$ mm Hg) without the INO. The mean ARDS-severity score of these patients prior to INO was 3.6 (in a range of 2.75-4.0). During daily brief interruptions of INO, pulmonary artery pressure and Q_{va} / Q_T (an index of venous admixture) were consistently increased and the PaO_2 / FiO_2 was consistently decreased. Extravascular lung water showed a declining trend and MHB levels always remained below 1.3 %. Conclusion: The data suggested that prolonged INO remained effective in reducing pulmonary artery hypertension and improving oxygen exchange without causing tachyphylaxis during 3-53 day period.

Summary and Conclusions:

This NDA proposes to use Inhaled nitric oxide, 5-80 ppm, as a locally administered pulmonary vasodilator in the treatment of PPHN.

The sponsors have submitted a few preclinical studies and have mostly abstracted published literature to suggest that inhaled NO has beneficial effects in animal as well as in clinical studies. The sponsor is aware of the intrinsic toxicity of NO and has made some effort to determine the safety of inhaled NO therapy. However the scope of this determination appears somewhat limited. In specific terms, the submission attempts to identify the INO dosages in animals that are fatal, nonfatal and those which may or may not invoke pathological changes. Although these "yes" and "no" answers are useful to some extent, they fall short in trying to identify the risk factors. At one dose or the other, NO is indeed toxic and therefore there appears an extra burden to prove, beyond reasonable and customary level of assurance, that the therapy is efficacious and safe. From the data submitted, there is little doubt that the test drug is efficacious. The question is at what level of risk. There are no easy answers because not only the test drug is toxic, genotoxic, mutagenic and perhaps neurotoxic, but there are multiple pathways of such toxicities from which only the MHB route is identified. Instead of being repetitious in trying to determine which dose is fatal and which is not, the specific studies on the mechanism of toxicity of NO would have helped more to generate the enthusiasm that is needed to assure the safety.

This research is further complicated because the therapy is targeted for a disease, PPHN of the newborns, the pathogenesis of which is poorly understood. One can still deal with (but not overcome) this concept of "a blind leading a blind", so to say, if there was an animal model which closely resembled PPHN of the newborns. Unfortunately, there is none. The value of preclinical data in projecting a "risk" depends very heavily on the use of appropriate experimental model which can replicate human disease as closely as possible. Obviously, if the treatment is intended for sick infants at high risk, the data obtained from healthy, adult animals will have a bearing which is , at the best, indirect. Although most of the data of this submission are "indirect" in this regard, the studies conducted in piglets and newborn lamb provide the much needed support on efficacy.

The following is the summary of studies submitted in this NDA:

Physiological, Pharmacological and Toxicologic effects of inhaled NO were investigated in anesthetized, instrumented dogs. Six hour inhalation of 80 - 640 ppm NO caused multiple electrocardiographic changes and death of the beagles which inhaled 640 ppm NO. The data suggested ventricular irritability and sinus tachycardia of dogs exposed to NO. The earliest physiological response was in the production of MHB. Since these dogs were anesthetized and catheterized, a subsequent study was

planned to determine if these effects persisted in unanesthetized instrumented dogs.

Unanesthetized, "normal" dogs were used in a follow-up study. With an exception of one dog (with a previous cardiac abnormality), INO up to 320 ppm did not affect cardiac conduction, rate or rhythm in conscious, spontaneously breathing dogs. Blood MHB level resulting from 40 ppm exposure was similar to control air exposure and therefore lower dosages may have little or no risk. The premature ventricular depolarization observed in the previous experiment were probably due to experimental condition and not due to the effects of INO.

The rate of MHB formation was determined in the plasma samples of the same anesthetized, instrumented dogs described above. The rate of MHB formation was stated at 0.0129 % / min. As stated in the preceding, the sponsor told us to disregard the electrocardiographic changes because they were due to anesthesia and instrumentation. Thus, the rate of MHB formation stated here can not have any meaning because the same artefact may be applicable to this determination. In a "by the way" kind of a statement, the rate of MHB formation in human volunteers was quoted at 0.0048 % / min to which I am unable to attach any significance.

Review Summary on Toxicology Data:

Toxicity effects of INO were investigated in SD rats exposed for 6 hrs/day for periods of 1, 3, or 7 consecutive days at 0-500 ppm NO. There were no mortalities up to 200 ppm NO but all the animals died at 300, 400 and 500 ppm NO. Even at the lowest dose of 80 ppm, the MHB concentration appeared to increase by 21%. Although the sponsor attributed the toxicity of NO totally to the formation of MHB, the support to that statement was lacking because none of the other, dozen or so, excitatory agents (histamine, prostaglandins, bradykinin, SRS etc) thought to act as humoral mediators for the onset/reversal of pulmonary inflammation were measured. Contrary to the interpretation of the sponsor, I think the formation of MHB is almost instantaneous irrespective of dosage (theoretically, it would be surprising if it is not) and it was quite apparent even at the lowest tested dose of 80 ppm.

Histopathological examination of the above rats revealed no insult to the respiratory tissues. It is somewhat troublesome that the respiratory tract was unaffected despite a 100% mortality, particularly, in high dose groups. It is conceivable that light microscopy is unable to detect these changes. When the same tissues were examined with Electron microscopy, intersticial edema was reported as an early change. It was moderate in animals exposed for 1 day at 200 ppm and mild/slight in those exposed for 7 days. This observation indicated transient oxidative injury to the respiratory epithelia. Most frustrating part of this experiment is in the fact that they examined the tissues of rats treated at 200 ppm but not those treated with 80 ppm. In the absence of a detectable injury, the examination of 80 ppm group would have generated much needed support for the therapeutic dosages proposed in this submission.

The results were qualitatively similar when the rats were exposed to NO inhalation for 28 days. Although there were no deaths in 40, 80 & 160 ppm groups, the MHB formation was quite apparent even at 40 ppm INO. Rapid respiration, ataxia, lethargy and blue & pale skin texture were noteworthy clinical observations which, according to the sponsor, were primarily limited to the 200 & 250 ppm exposure groups. The sponsor tends to imply that since there were no deaths, the low dosages (40 to 160 ppm) "are" safe. Unfortunately, the data seems to suggest to me that the rat is able to detoxify the MHB formed between 40 - 160 ppm without causing a fatality. How this will translate into sick human infants still remains unknown. Ultrastructural effects of NO exposure were investigated in bronchiolar, transitional and respiratory tissue samples of the animals of the foregoing toxicology study. There were a number of changes in the morphology but these changes were also found in the controls and therefore the sponsors stated that the oxidative insult to respiratory epithelia may be low/minimal at 200 ppm.

The following are my comments on specific issues:

Efficacy:

A number of studies suggest that low doses (5-80 ppm) of inhaled NO may act as a selective pulmonary vasodilator reversing both hypoxia and pulmonary hypertension in experimental animals. I fully agree. Although there are suggestions that the benefits of this therapy may be dose related, at the present stage of knowledge it appears prudent to rely more in the findings of Etches et al (1994) who found that there was no significant difference noted between the various doses of nitric oxide and that a consistent maximal effect obtained at a minimum dose 5 ppm was statistically no better than that obtained with 80 ppm INO in the newborn piglets.

Tolerance and Withdrawal: (as requested by the division)

A study in the literature by Frostell et al (1991) indicated that Tolerance to nitric oxide was not evident in the vasodilatory effect of NO or the MHB levels in awake lambs. Because NO and its metabolites may have a very rapid turnover and the fact that the therapy is intended for short durations, it appears that tolerance may not be an issue.

In a clinical study, Rossaint et al, (1993) found that prolonged INO remained effective in reducing pulmonary artery hypertension and improving oxygen exchange without causing tachyphylaxis during 3-53 day period. Despite limited information, theoretical consideration exclude the possibility of withdrawal problems.

Rebound of PAH: (as requested)

I was unable to identify animal studies addressing this issue. Only in one paper, Atz et

al (Ann. Thoracic Surg., 62, 1759 - 1764, 1996) studied this issue in infants with pulmonary hypertension after surgical repair of total anomalous pulmonary venous connection. Inhaled NO selectively vasodilated all patients with pulmonary hypertension. Withdrawal of NO after prolonged inhalation was associated with transient rebound in pulmonary hypertension that dissipated within 60 mins. Thus, it seems that the rebound of PAH is very likely to subside, at the most, after a repeat treatment.

INO-related dysfunctions:

i) Surfactants:

Hillman et al (1996) studied surfactant dysfunction in rats after inhalation of nitric oxide. The investigators found surfactant dysfunction caused by inhaled NO which was thought to be due to alteration of proteins in epithelial lining. However the doses used in these studies were high. Low doses and shorter duration of INO may not invoke this dysfunction and thus may not be an immediate concern.

ii) Bleeding :

In a letter published in Lancet, Hogman et al (1993) reported prolongation in bleeding time after NO administration in rabbits and man. Once again this risk may be minimum if the proposed INO therapy is adhered to at low doses / shorter durations as recommended here. Nonetheless, the awareness of this dysfunction merits listing in the package insert.

Toxicity:

Toxicity due to INO: At one dose or the other, inhaled nitric oxide is toxic. Animal studies presented here and those published in the literature have attested to this fact and attempted to differentiate non-lethal doses from the lethal. Depending upon the species, the age and the health status of experimental animal, a variety of the so called "non-lethal" dosages can be identified. However, this data is unable to project safety because none of these "non-lethal" doses were ever tested in animals which specifically replicate PPHN of human infants. Thus, in the case of this drug, the animal data appears unable to project safety.

There appears a general consensus that one of the many pathways of NO-toxicity is via the formation of MHB intermediate. There was no apparent lung damage at lethal doses of NO. The animals died due to high MHB and cyanosis. Our division had suggested to the sponsor to infuse methylene blue to correct MHB and then see how high one could go with NO inhalation. The experiment would have helped to identify toxicity not related to MHB but it was not done.

I am not convinced from the data of this submission that there was any dose, whatsoever, where there was no net formation of MHB. In fact, such a low dose may not exist in view of the high affinity of NO for HB. Thus, in the case of NO, the non-lethal dose perhaps predicts the tolerance of toxicity (due to MHB) by rat (or any other animal) rather then project safety. Since it is unknown how a non-lethal dose (found, eg, in rat) will affect an already sick human infant, it is very difficult to generate the level of enthusiasm that is needed in support of this therapy.

Toxicity due to possible formation of NO_2: (as requested)

There is overwhelming concurrence, in fact without exception, that NO_2 is a highly toxic gas. The sponsors also acknowledge. However, their argument is that the device, they have designed, may substantially reduce the formation of NO_2 . Although it very well may, it is impossible to rule out mechanical and/or human errors which can plague the flawless function expected of the device.

Dr. Lipicky wanted us to comment on the limit of NO_2 toxicity. There are a number of recommendations from OSHA, NIOSH, EPA, NAS and ACGIH on the safety limits. I am quoting the following from the search made by my colleague, Dr. Tom Papoian, who deserves all the credit for this indepth search, if any additional is due. The recommendation on safety levels vary from 1 ppm (National Academy of Science) to 40 ppm (US Dept. of Interior, Bureau of Mines) depending upon the nature of task assigned to the respective agency. Furthermore, there are "prudent" recommendations and then there are "flawed" recommendations as adjudged by the US district courts. Nonetheless, there appears a consensus from three respectable agencies (National academy of Sciences, OSHA and NIOSH) on the limit of 1 ppm NO_2, with which I tend to agree. If the sponsors can assure that there will never be NO_2 to exceed 1 ppm, they might well be within reason; assuming that there are assurances for the device.

Genotoxicity:

Positive genotoxic potential of NO have been demonstrated in several test systems including Ames test and human lymphoblasts. Three possible mechanisms of genotoxicity have been proposed. First, NO can produce N-nitroso compounds which can alkylate DNA. Second, direct reaction with the primary amino groups of DNA resulting in deamination. Third, NO may form highly oxidative peroxinitrite and hydroxy radical which can cause oxidative damage to DNA. Even if one agrees with the sponsor that the genotoxicity may arise only with higher doses of longer duration, it is not known whether it is applicable to infants suffering from PPHN.

Recommendations:

The preclinical animal data of this submission is unable to completely assure safety primarily because a) all the mechanisms of NO toxicity are not known and b) the data falls short in proving beyond reasonable doubts that NO can be administered at a safe dose which does not, *per se,* form MHB. Although MHB can be monitored and corrected with methylene blue as needed, information is not available on toxicity not related to MHB. Although the device proposed by the sponsor may minimize the risk due to NO_2 , the risk can not be excluded because of possible mechanical and human failures. Because NO_2 is highly toxic, it is critical to exclude this risk in total. On the other hand, the possibility that the therapy may save a life of a very sick infant is very compelling, indeed, to search for any clue, whatsoever, which can provide even a little more assurance on safety.

If the clinical data suggest distinct benefits (see the review of Medical officer) of very low doses of INO in PPHN at a tolerable risk, I tend to favor the use of a very low dose, eg 5-10 ppm, of NO. There is a support from the animal data on the efficacy for the low dose and although the risk can not be excluded, it might be minimum. The dose should never exceed 10 ppm because there may not be any improvement at higher doses. Of course, the assessment of benefit / risk projected by the clinical data supersede this recommendation.

/S/

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