6.3 Chronic Pulmonary Injury (cont)

Review of Systems/Illnesses During Follow-up in INO-01/ -02

The INO-01/ -02 trials also performed a review of systems at the follow-up visit. Note that while the use of home O_2 occurred only in the I-NO groups, there was no detectable differences in the occurrence of other pulmonary disease. Fewer patients in the I-NO group were reported to have had severe URIs.

Table 6.3.6 Pulmonary review of systems for the infants seen in follow-up from the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Home Oxygen	0 (0%)	8 (22.2%)	1 (3.4%)	5 (16.1%)	14 (14.6%)
Mean age when O_2 was D/C'd (months)[b]	–	4.0±3.0	1.0±0.0	3.3±1.3	3.5±2.5
Asthma	5 (13.9%)	7 (19.4%)	3 (10.3%)	2 (6.5%)	12 (12.5%)
Bronchiolitis	4 (11.1%)	7 (19.4%)	4 (13.8%)	2 (6.5%)	13 (13.5%)
Bronchitis	2 (5.6%)	4 (11.1%)	3 (10.3%)	2 (6.5%)	9 (9.4%)
Pneumonia	3 (8.3%)	3 (8.3%)	3 (10.3%)	2 (6.5%)	8 (8.3%)
Severe URI	11 (30.6%)	8 (22.2%)	6 (20.7%)	6 (19.4%)	20 (20.8%)

a. Data from NDA vol. 9.3, Tables 11.

b. For infants who received O_2 at time of initial discharge.

In conclusion, the data regarding the long-term pulmonary toxicity of I-NO is conflicted, and depends on the trial data used. In the INO-01/ -02 trial, more infants were taking pulmonary medications at time of follow-up, and used O_2 after discharge. In the CINRGI trial, the trends for chronic lung injury instead favor I-NO. NINOS appears to be neutral with respect to the occurrence of chronic pulmonary injury. Whether this scatter is a result of the imprecise tools being used to identify pulmonary disease (medicines used, use of O_2) or the result of differences in the trials (e.g., dose of I-NO, duration of administration, patient populations) simply cannot be determined with any certainty in these small datasets. In aggregate, the data do not allow us to conclude that there is either a salutary or adverse effect of I-NO on chronic pulmonary disease, but it also does not exclude the occurrence of either effect in susceptible (and undefined) populations. Additional information is necessary.

6.4 Acute Neurological Injury

CINRGI

The CINRGI trial collected radiological information about the neurologic changes seen in a subset of the population. In that subset, a higher fraction of the I-NO group had abnormal CT scans reported. No difference in the rate of abnormal neurologic examinations was detected.

Table 6.4.1 Discharge neurologic status in the CINRGI trial[a].

	Placebo	I-NO
Abnormal Head U/S	12/52 (23.1%)	5/42 (11.9%)
Abnormal Head CT	8/34 (23.5)	12/25 (48.0%)
Abnormal Neurologic Exam	8/41 (19.5%)	7/48 (14.6%)
Abnormal CT, U/S or Neurologic Exam	19/89 (21.3%)	17/9 (17.5%)

a. Data from CINRGI study report, table 67. p Values per sponsor.

NINOS

No differences between treatment groups in the incidence of seizures or other markers of acute neurologic changes were noted in the NINOS trial.

Table 6.4.2 (from 6.0.1.13.1.2) Comparison of specific safety parameters during the NINOS trial[a].

Neurologic Adverse Events	Placebo Group (n=121)	I-NO Group (n=114)
Seizures requiring therapy	20/122 (17%)	13/114 (11%)
Brain Infarct	4/82 (5%)	7/77 (9%)
Interventricular hemorrhage (IVH)[a]	21/108 (19%)	16/111 (14%)
IVH Grade I	10/21 (62%)	9/16 (56%)
IVH Grade II	3/21 (14%)	0/16 (0%)
IVH Grade III-IV	8/21 (38%)	7/16 (44%)
Periventricular leukomalacia	3/82 (4%)	4/77 (5%)

INO-01/ -02

The table below summarizes the results of the specified safety parameters measured at the end of hospitalization or 28 days in the INO-01/ -02 trial. There were no significant differences between control and I-NO groups for any of the endpoints. Note that not all subjects have data for a given parameter.

Table 6.4.3 (from 6.0.3.13.1.1) Neurologic disease in INO-01/ -02.[a]

Changes in safety endpoints	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Incidence of seizures	7/41 (17%)	5/40 (12%)	10/35 (28%)	7/37 (19%)	22/112 (20%)
Incidence of sensorineural hearing loss[b]	5/36 (14%)	3/38 (8%)	6/29 (21%)	7/31 (23%)	16/98 (16%)
Abnormality on cranial ultrasound[b]	4/28 (14%)	3/27 (11%)	3/23 (13%)	2/21 (10%)	7/71 (10%)
Intracranial hemorrhage or infarct detected by ultrasound[c]	1/28 (4%)	0/27 (0%)	1/23 (4%)	0/21 (0%)	1/71 (2%)
Abnormality on CT or MRI scan of head[d]	9/18 (50%)	2/15 (13%)	8/19 (42%)	4/11 (36%)	14/45 (31%)
Interventricular hemorrhage	2/18 (11%)	0/15 (0%)	0/23 (0%)	0/11 (0%)	0/45 (0%)
Periventricular hemorrhage	0/18 (0%)	0/15 (0%)	1/23 (5%)	1/11 (9%)	2/45 (4%)
Intracranial hemorrhage[e]	1/18 (6%)				2/45 (4%)
Periventricular leukomalacia	0/18 (0%)	0/15 (0%)	1/23 (5%)	1/11 (9%)	2/45 (4%)
Extensive cytotoxic edema	0/18 (0%)	0/15 (0%)	0/23 (0%)	1/11 (9%)(	1/45 (2%)
Subdural hematoma	0/18 (0%)	0/15 (0%)	1/23 (5%)	0/11 (0%)	1/45 (2%

a. The sponsor identified the changes in methemoglobin and NO_2 levels, along with overall adverse events, as the most important markers.

b. Only those infants who had a normal cranial ultrasound at the start of the trial and an ultrasound at the end of the trial are included.

c. Category includes one subject with suspected white matter hemorrhage, one grade one germinal matrix hemorrhage, and one infarct, detected by ultrasound. Only subjects with normal baseline ultrasound were included.

d. Abnormalities detected at any time during the hospitalization. No baseline scans are available in most cases, making it difficult to date the onset of the abnormality.

e. Category includes parietal lobe, posterior fossa and frontal lobe hemorrhages.

The INOSG trial did not collect information about neurological injury.

In conclusion, the data do not suggest an effect of I-NO on the incidence of neurologic injury during the initial hospitalization when I-NO was administered.

6.5 Chronic Neurological Injury

In this discussion, and in the discussion of chronic pulmonary injury, it is essential to remember that the degree of follow-up for each of the trials was <90% for almost all endpoint, and <50% for some endpoints of interest. This will be apparent by comparing the numbers in the denominators of the results with the number of patients enrolled in each of the trials. This obviously introduces potential biases (both positive and negative) in the interpretation of the results.

CINRGI

The CINRGI trial collected 6- and 12-month data from a fraction of the population. Recall that there were 89 infants in the control group and 97 in the I-NO group. The results are of limited interpretability.

Table 6.5.1 Six-month follow-up data from the CINRGI trial[a].

	Placebo	I-NO
Abnormal Neurologic Exam-6 Month	7/38 (18.4%)	9/32 (28.1%)
Abnormal Neurologic Exam- 12 Month	0/24 (0%)	3/20 (15.0%)

a. Data from CINRGI study report, table 38-39.

NINOS

NINOS enrolled 121 placebo patients and 114 I-NO patients. No significant differences were detected with regard to any chronic neurologic abnormalities in the NINOS trial. In data not shown here, assessments of mental development (Bayley's), psychomotor development and audiology were similar in both treatment groups. A lower incidence of seizures at follow-up was noted in the I-NO group.

Table 6.5.2 Neurologic diagnoses for the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Normal	69 (79.3%)	66 (77.6%)
Global hypotonia	3 (3.4%)	0 (0%)
Monoplegia	2 (2.3%)	2 (2.4%)
Diplegia	3 (3.4%)	2 (2.4%)
Hemiplegia-right side	1 (1.1%)	2 (2.4%)
Quadraplegia	5 (5.7%)	4 (4.7%)
Truncal hypotonia	4 (4.6%)	4 (4.7%)

a. Data from NDA vol. 11.1, Table 52.

Table 6.5.3 Cerebral palsy and seizures in the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Cerebral palsy present	9 (10.3%)	10 (11.9%)
Mild or Moderate Cerebral Palsy	4 (4.6%)	5 (6.0%)
Severe Cerebral Palsy	5 (5.7%)	5 (6.0%)
Seizures present	13 (14.9%)	4 (4.7%)

a. Data from NDA vol. 11.1, Table 53.

INO-01/ -02

The INO-01/ -02 trial collected data on the use of anticonvulsants at the end of 1 year follow-up for the 41 placebo and 104 I-NO patients. Few infants were using anticonvulsants at the time of follow-up--two individuals in the I-NO 20 ppm group.

Table 6.5.4 Post-discharge medications at the one-year follow-up visit in the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Anticonvulsants	0 (0%)	0 (0%)	2 (6.9%)	0 (0%)	2 (2.1%)

a. Data from NDA vol. 9.3, Tables 9.

6.5 Chronic Neurological Injury (cont)

Review of Systems/Illnesses During Follow-up in INO-01/ -02

A review of systems performed at the follow-up visit found relatively few problems. The respiratory ROS is summarized separately. The occurrence of abnormalities in the neurologic Review of Systems is summarized below. Reports of strabismus were more common in the I-NO groups.

Table 6.5.5 Review of systems for the infants seen in follow-up from the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Strabismus	1 (2.8%)	1 (2.8%)	6 (20.7%)	4 (12.9%)	11 (11.5%)
Hearing problems	3 (8.3%)	5 (13.9%)	3 (10.3%)	1 (3.2%)	9 (9.4%)
Speech problems	4 (11.1%)	5 (13.9%)	6 (20.7%)	4 (12.9%)	15 (15.6%)

a. Data from NDA vol. 9.3, Tables 10.

The sponsor also collected data on the occurrence of seizures in the follow-up population. The only infants with seizures were in the 20 ppm and 80 ppm I-NO groups. There were, however, no differences noted in the incidence of abnormal neurologic examinations at 1 year.

Table 6.5.6 Incidence of seizures and neurologic abnormalities at follow-up in the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Seizures Present	0 (0%)	0 (0%)	4 (13.8%)	3 (9.7%)	7 (7.3%)
Cerebral Palsy Present	2 (5.6%)	0 (0%)	4 (13.8%)	3 (9.7%)	7 (7.3%)
Neurologic Abnormalities on Physical Exam					
None	28 (77.8%)	31 (86.1%)	20 (69.0%)	23 (74.2%)	74 (77.1%)
Mild	3 (8.3%)	1 (2.8%)	2 (6.9%)	1 (3.2%)	4 (4.2%)
Moderate	4 (11.1%)	3 (8.3%)	5 (17.2%)	5 (16.1%)	13 (13.5%)
Missing	1 (2.8%)	1 (2.8%)	2 (6.9%)	2 (6.5%)	5 (5.2%)

a. Data from NDA vol. 9.3, Table 16.

Finally, in data not shown here (see INO-01/ -02 update elsewhere in this document), the incidence of abnormalities in mental development, psychomotor development and audiology were assessed at follow-up. No worrisome patterns were evident in the data obtained from those patients with available follow-up.

In conclusion, the data available do not reveal a clear pattern of long-term neurologic adverse outcomes following I-NO therapy. In data not shown here, assessments of mental development (Bayley's), psychomotor development and audiology were similar in both treatment groups from the INO-01/ -02 and NINOS trial follow-up data (sections 4.1 and 4.2 of this review).

The increased incidence of seizures reported in the INO-01/ -02 trial is countered by their decreased incidence in the I-NO group of NINOS. The increased frequency of strabismus was only assessed in the INO-01/ -02, and is difficult to interpret with the small numbers of patients. There is a striking increase in strabismus relative to placebo in both the 20 and 80 ppm groups, however, raising the possibility of an adverse effect. Further data are needed to address the issue of strabismus following I-NO use.

6.6 Laboratory Abnormalities

a. Increased Methemoglobin and NO_2 concentrations

CINRGI

CINRGI had a maximum dose of 20 ppm I-NO for the first 4 hours, after which time the infants were reduced to 5 ppm as tolerated.

Elevated Methemoglobin levels

Two infants in the I-NO group had methemoglobinemia >4% during the treatment period (1.9% of the infants exposed to I-NO). No control infant had elevated methemoglobin. The I-NO group also had a higher mean methemoglobin level during the treatment period on average, when compared with the control group (p = 0.001 per sponsor).

Elevated NO_2 Levels

No infant in either treatment group developed NO_2 levels >5 ppm during the study. Likewise, there was no significant difference between the two treatment groups with regard to the changes in mean NO_2 levels during the treatment period (p=0.83).

NINOS

NINOS used an initial dose of 20 ppm I-NO. If the infant failed to respond with an increase in PaO_2, the I-NO could be increased to 80 ppm.

Elevated Methemoglobin levels

A total of 11 subjects (4 controls, 7 I-NO) had their study gas decreased because their methemoglobin levels were >5%. All continued on study gas at lower flow rate. No subject was discontinued because of NO_2 >7 ppm or methemoglobin >10%.

Table 6.6.1 (from 6.0.1.13.2b.1) Peak Methemoglobin levels from the NINOS trial.

Changes in safety endpoints	Control	Combined I-NO	p value
Peak methemoglobin level during first 12 hours of study gas	1.0±0.6	2.0±1.5	<0.001
Peak methemoglobin level at any time	1.2±0.8%	2.4±1.8%	<0.001
Peak methemoglobin level at any time			
0.0 - 1.0%	52/112 (46%)	15/110 (14%)	<0.001
1.1 - 2.0	49/112 (44%)	49/110 (45%)	
2.1 – 30	6/112 (5%)	23/110 (21%)	
3.1 - 5.0	4/112 (4%)	12/110 (11%)	
5.1 to 10	1/112 (1%)	11/110 (10%)	
Peak methemoglobin level at any time, excluding 8 subjects who received wrong study gas	1.2±0.8%	2.4±1.8%	<0.001

Elevated NO_2 Levels

Only one individual had a NO_2 level >7.0 % during the trial (subject #A08 from center 55). The level was 9.1, and the subject underwent a successful wean of study gas.

Table 6.6.2 (from 6.0.1.13.2a.1) Peak NO_2 levels in ppm from the NINOS trial.

Changes in safety endpoints	Control	Combined I-NO	p value
Peak NO_2 level during first 12 hours of study gas	0.1±0.3	0.6±0.9	<0.001
Peak NO_2 level at any time	0.1±0.3	0.8±1.2	<0.001
Peak NO_2 level at any time			
0.0 - 1.0	98/101 (97%)	85/110 (77%)	<0.001
1.1 - 3.0	3/101 (3%)	21/110 (19%)	
3.1 - 5.0	0/101 (0%)	2/110 (2%)	
5.1 - 7.0	0/101 (0%)	1/110 (1%)	
7.1 to 10	0/101 (0%)	1/110 (1%)	
Peak NO_2 level at any time, excluding 8 subjects who received wrong study gas	0.0±0.3	0.8±1.2	<0.001

6.6 Laboratory Abnormalities (cont)

INO-01/ -02

INO-01/ -02 randomized the treatment group to receive 5, 20 or 80 ppm I-NO.

Methemoglobin levels

The average methemoglobin level was >1% at any time only in the 80 ppm I-NO group. After 12 hours of exposure to 80 ppm I-NO, the mean methemoglobin level peaked at 5.08±2.32%.

The average time to after starting I-NO to the development of elevated methemoglobin was 10.4±9.5 hours overall, but only 3 subjects developed elevated methemoglobin levels >10 hours after starting the I-NO. This is reflected in the median time to peak methemoglobin level, which was 8 hours. The I-NO was discontinued in four of the subjects and reduced and ultimately discontinued in the other 9 subjects.

In the INO-01/ -02 trial, the average peak methemoglobin level was significantly higher in the 80 ppm I-NO group. After 12 hours of exposure to 80 ppm I-NO, the mean methemoglobin level peaked at 5.08±2.32%.

Table 6.6.3 (from 6.0.1.13.2b.1) Peak methemoglobin levels from the INO-01/ -02 trial (not all subjects had data available).

Changes in safety endpoints	Control	I-NO 5 ppm	20 ppm	80 ppm	Combined I-NO
Peak methemoglobin level at any time	0.7±0.42	0.89±0.88	1.16±0.69	5.77±2.8	2.57±2.8
Peak methemoglobin level at any time					
0.0 - 1.0%	31/40 (78%)	28/40 (70%)	18/36 (50%)	0/37 (0%)	46/113 (41%)
1.1 - 2.0	9/40 (22%)	11/40 (28%)	17/36 (47%)	2/37 (5%)	30/113 (26%)
2.1 – 30	0/40 (0%)	0/40 (0%)	0/36 (0%)	4/37 (11%)	4/113 (4%)
3.1 - 5.0	0/40 (0%)	0/40 (0%)	1/36 (2%)	9/37 (24%)	10/113 (9%)
5.1 to 10	0/40 (0%)	1/40 (2%)	0/36 (0%)	18/37 (49%)	19/113 (17%)
>10.0	0/40 (0%)	0/40 (0%)	0/36 (0%)	2/37 (5%)	2/113 (2%)

Mean NO_2 levels in the INO-01/ -02 trial.

In the INO-01/ -02 trial, the average NO_2 level was >1% at any time only in the 80 ppm I-NO group. The large majority of subjects in the I-NO group had peak NO_2 concentrations >3.0 ppm. Note, however, that the peak NO_2 concentration for the 80 ppm group was significantly higher overall, and 7/9 subjects who had NO_2 levels >3.0 at any time during the trial were in the 80 ppm group.

Table 6.6.4 (from 6.0.3.13.2a.1) Peak NO_2 levels in ppm from the INO-01/ -02 trial.

Changes in safety endpoints	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
Mean peak NO_2 level at any time	0.59±0.8	0.53±.73	0.48±.62	2.6±1.2
Peak NO_2 level at any time (ppm)				
0.0 - 1.0	32/42 (76%)	34/41 (83%)	30/35 (86%)	0/37 (0%)
1.1 - 3.0	8/42 (19%)	6/41 (15%)	4/35 (11%)	29/37 (78%)
3.0- 5.0	2/42 (5%)	1/41 (1%)	1/35 (1%)	4/37 (11%)
5.1 - 7.0	0/41 (0%)	0/41 (0%)	0/35 (0%)	3/37 (8%)
7.1 to 10	0/41 (0%)	0/41 (0%)	0/35 (0%)	0 (0%)

6.6 Laboratory Abnormalities (cont)

INOSG

INOSG administered I-NO at a dose of 80 ppm.

NO_2 levels

No information on NO_2 levels was submitted as part of the NDA from the INOSG trial.

Methemoglobin levels

Peak methemoglobin levels averaged 3.8±4.0 during the first twelve hours of therapy, and 5.3±5.8 at any time during the trial for the subjects in the I-NO group with available data.

Three of the infants who received I-NO had peak methemoglobin levels >5% during the trial.

Table 6.6.5 (from 6.0.2.13.2b.1) Peak Methemoglobin levels from the INOSG trial.

Changes in safety endpoints	Control	Combined I-NO
Peak methemoglobin level during first 12 hours of study gas	N/A	3.8±4.0 (n= 11)
Peak methemoglobin level at any time	N/A	5.3±5.8 (n= 11)
Peak methemoglobin level at any time[a]		
0.0 - 1.0%	N/A	2/11 (18%)
1.1 - 2.0	N/A	2/11 (18%)
2.1- 4.0	N/A	2/11 (18%)
>4.0	N/A	5/11 (45%)

a. Eleven of 16 subjects who received I-NO, and none of the control subjects, have data.

In conclusion, the incidence of both methemoglobinemia and elevated NO_2 concentrations is markedly increased in the 80 ppm groups of the INO-01/ -02 trial and increased NO_2 was seen in the I-NO group of the INOSG trial. In distinction, in the three trials using 20 ppm, 15/243 (6.2%) infants on I-NO had a methemoglobin level >3.0%, the majority of them in the NINOS trial, following increase in I-NO from 20 to 80 ppm . The incidence of elevated NO_2 levels in the 20 ppm group was 3/2443 (0.1%) of the infants, much lower than the rate at 80 ppm I-NO. This result should be put in context with the efficacy data from the NINOS trial (see original NDA submission, vol. 2.14, page 92). Per the sponsor's analysis, an individual who failed to respond to I-NO 20 ppm was also failed to respond to 80 ppm. No data are available about the effect of doses between 20 and 80 ppm in the same population. These data argue for the use of no more than 20 ppm for two reasons: 1) use of 80 ppm is associated with undesirable side-effects, and 2) infant who do not have improved oxygenation on 20 ppm are unlikely to respond to 80 ppm.

6.6 Laboratory Abnormalities (cont)

b. Eosinophilia

INO-01/ -02

In the INO-01/ -02 study, there was a trend towards a higher mean eosinophil count in the subjects who received I-NO, especially the 80 ppm group

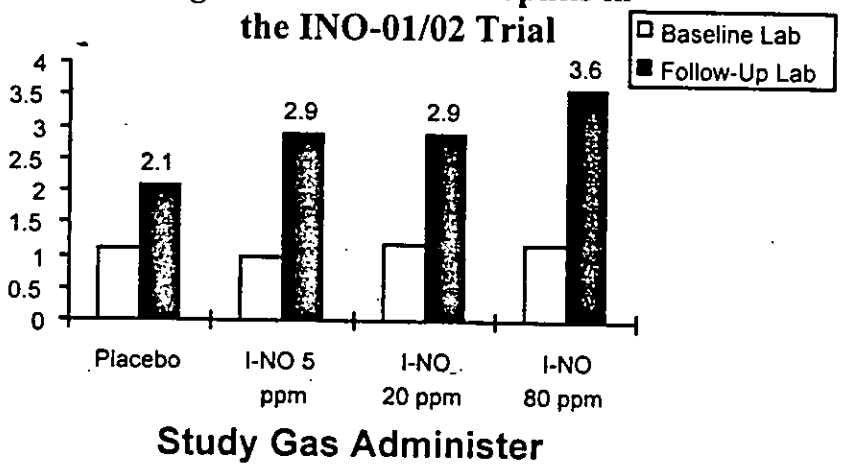
Table 6.6.6 (from 8.2.3.2.5) Mean changes in eosinophil counts from INO-01/ -02[a,c].

	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO
Eosinophils (% WBCs)	1.1 ±1.8 n=41	2.1 ±2.2 n=36	1.0 ±1.3 n=41	2.9 ±3.2 n=39	1.2 ±2.3 n=36	2.9 ±3.0 n=32	1.2 ±1.5 n=35	3.6 ±5.1 n=36

a. Source: NDA volume 2.50, pages 341010-341510 and volume 2.25.
b. Per protocol, after values to be taken no more than 12 hours after end of exposure to treatment gas.
c. Data shown as mean±standard deviation (# of subjects with data).

This same data is presented as a graph below.

Figure 8.2.3.2.6 Eosinophils in the INO-01/02 Trial



There were also subjects who had a markedly abnormal number of eosinophils after exposure to I-NO, identified through examination of the individual laboratory records.

Table 6.6.7 (from 8.2.3.2.7) Individuals with markedly abnormal post-study gas eosinophil counts from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline Value	Post-I-NO value	Notes on F/U
Placebo				
01-08001	Eosinophils	1	8	Discharge without ECMO
I-NO 5 ppm				
01-10002	Eosinophils	0	14	No discharge data available
01-11012	Eosinophils	0	7	Died without ECMO of progressive hypoxia
I-NO 20 ppm				
03-67002	Eosinophils	1	17	Discharged without ECMO, with seizures
I-NO 80 ppm				
02-11007	Eosinophils	5	22	Discharged after ECMO, with seizures and chronic lung disease (CLD)
01-05005	Eosinophils	2	9	Discharged without ECMO, no seizures or CLD
02-15006	Eosinophils	1	11	Discharged without ECMO, no seizures or CLD

a. Data from NDA, volume 2.25, individual patient listings and table 8.1.6.2.2.1a.1.
b. Lab tests identified as markedly abnormal were >2X upper limits of normal.

CINRGI

In CINRGI no such trend was evident. There was a marked increase in the number of patients with eosinophils >5% in both treatment groups, however, evident between days 0 and 2.

Table 6.6.8 Number of patients with eosinophils >5%[a].

Days	Placebo	I-NO
Baseline	6 (11%)	2 (3%)
1	13 (26%)	13 (24%)
2	20 (40%)	33 (39%)
3	19 (42%)	16 (37%)
4	23 (55%)	23 (54%)

a. Data from table A-15, CINRGI study report.

NINOS, INOSG

These trials did not collects hematology data.

In conclusion, the data do not suggest an effect of I-NO to increase eosinophil counts, although they do suggest that a process common to the infants in both treatment arms that is associated with the development of eosinophilia.

c. Abnormal LFTs

A normal part of a safety review is an examination of the database for evidence of liver toxicity, as judged by increases in liver function tests. Labs were only collected in the CINRGI and INO-01/ -02 trials, limiting the available data.

CINRGI

The CINRGI trial collected data on the incidence of marked elevations of bilirubin and SGPT as well as the mean changes in both labs. In data not shown, the mean changes were similar between the treatment groups. For the incidence of marked elevations, the data, summarized below, do not suggest an increased incidence of elevations in the I-NO group. The number of patients with available data (shown for each data point), however, is quite limited. Note also the high percentage of patients with abnormally-elevated AST at baseline.

Table 6.6.9 Elevations of Direct Bilirubin to >1 mg/dl[a].

Day	Placebo	I-NO
Baseline	1/12 (8.3%)	1/12 (8.3%)
1	2/38 (5.3%)	3/28 (10.7%)
2	5/37 (13.5%)	4/31 (12.9%)
3	7/31 (22.6%)	9/30 (30%)

a. Data from CINRGI study report, table A-25.

Table 6.6.10 Elevations of AST to >70 U/l[a].

Day	Placebo	I-NO
Baseline	18/39 (46.2%)	27/50 (50.9%)
1	11/34 (32.4%)	11/29 (37.9%)
2	10/27 (37.0%)	12/31 (38/7%)
3	10/21 (47.6%)	6/19 (31.6%)

a. Data from CINRGI study report, table A-25.

6.6 Laboratory Abnormalities (cont)

INO-01/ -02

The interpretation of I-NO effects on LFTs in the INO-01/ -02 study is complicated four things.

1) the high incidence of abnormal LFTs at baseline. The table below shows the number of subjects with abnormal labs at baseline in the INO-01/ -02 trial, including high percentages of both alkaline phosphatase and LDH abnormalities at baseline.

Table 6.6.11 (from 8.2.2.1.2) Number of subjects with normal baseline LFTs from INO-01/ -02[a,c].

Laboratory	Control n =38	I-NO 5 ppm n = 45	I-NO 20 ppm n =41	I-NO 80 ppm n = 37
Alkaline Phosphatase	16/34 (47%)	12/32 (38%)	19/29 (66%)	19/29 (66%)
LDH	2/28 (7%)	5/31 (16%)	1/25 (4%)	2/29 (7%)
AST[b]	8/33 (24%)	7/32 (22%)	5/29 (17%)	6/28 (21%)
Total Bilirubin	19/34 (56%)	22/39 (56%)	16/32 (52%)	19/32 (59%)

a. Data from original NDA volume 2.18, Table T-21.

b. SGOT (serum glutamate pyruvate transaminase) = AST (aspartate transaminase); SGPT (serum glutamic-oxaloacetic transaminase) or ALT (alanine transaminase); GGT (gamma-glutamyl transferase).

c. No data was collected during the INO-01/ -02 and /-03 trials on GGT or SGPT levels.

2) the absence of data on changes in ALT (serum glutamic-oxaloacetic transaminase) and GGT(gamma-glutamyl transferase).

No data was collected during the CINRGI or INO-01/ -02 and /-03 trials on ALT or GGT levels. This limits the ability of this database to detect hepatocellular injury largely to detected changes in AST (in the context of altered alkaline phosphatase, LDH, and total bilirubin, which were collected).

3) the lack of available follow-up for abnormal labs.

As discussed above, two sets of labs were collected, and no follow-up labs are available for abnormalities identified on the second set.

4) the changing normal ranges for individual labs shortly after birth.

The normal values for some labs (total bilirubin in particular) change from day to day in the early neonatal period. Labs were deemed normal or abnormal depending on the limits associated with each individual lab sample and subject.

Overall, the mean LFTs tended to fall from baseline to post-INO[b]. In all groups except I-NO 20 ppm, alkaline phosphatase fell significantly. Mean values for LDH and AST also fell, but the differences were not significant.

Table 6.6.12 (from 8.2.2.2.3) Mean LFT values from INO-01/ -02[a,b,c].

Lab Test[c]	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO
Alkaline Phosphatase	302.8±313 n=40	164±206 n=34	465±581 n=38	175±285 n=34	353±552 n=36	155±217 n=30	366±514 n-34	141±92 n=33
LDH	1617 ±1519 n=38	1069 ±1275 n=32	1479 ±1096 n=36	1134 ±937 n=33	3060 ±6615 n=32	1218 ±1783 n=29	1976 ±2160 n=34	1338 ±1271 n=31
AST	109 ±101 n=39	69 ±72 n=34	121 ±89 n=38	64 ±53 n=34	312 ±760 n=35	81 ±86 n=30	258 ±584 n=34	78 ±61 n=31
Total Bilirubin	4.8 ±3.1 n=41	5.0 ±4.8 n=35	4.2 ±2.7 n=40	5.3 ±4.9 n=40	5.0 ±3.6 n=36	6.8 ±6.3 n=31	4.6 ±3.2 n=35	5.1 ±3.4 n=34

a. Source: NDA volume 2.50, pages 341010-341510 and volume 2.25.

b. Per protocol, follow-up labs were to be taken no more than 12 hours after end of exposure to treatment gas.

c. Data shown as mean±standard deviation (# of subjects with data). Shaded boxes indicate that baseline and post-study gas labs differ significantly using 2-sided unpaired t test.

In the database from INO-01/ INO-02 and -03, newly-abnormal AST occurred in 1 control subject (2%) and in 3 I-NO subjects (2%). AST values which became more abnormal, including those who started with abnormal baselines, occurred in 5% of control subjects and 11% of I-NO subjects. The table below shows the number of subjects in each I-NO group. The numbers are too small to infer a relationship between I-NO dose and AST abnormalities.

6.6 Laboratory Abnormalities (cont)

Table 6.6.13 (from 8.2.2.2.4) Abnormal LFTs from INO-01/ -02 and INO-03[c].

	Control n =38	I-NO 5 ppm n = 45	I-NO 20 ppm n =41	I-NO 80 ppm n = 37	I-NO combined n = 123
Elevated Total Bilirubin					
New abnormalities[a]	1 (3%)	4 (9%)	1 (2%)	1 (3%)	6 (5%)
Values >12	3 (8%)	4 (9%)	5 (12%)	3 (8%)	12 (10%)
Elevated AST					
New abnormalities[a]	1 (3%)	1 (2%)	0 (0%)	2 (5%)	3 (2%)
New or worsening abnormalities[b]	2 (5%)	6 (13%)	2 (5%)	6 16%)	14 (11%)
Elevated Alkaline Phosphatase					
New abnormalities[a]	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (<1%)
New or worsening abnormalities[b]	0 (0%)	2 (4%)	0 (0%)	0 (0%)	2 (2%)

a. These subjects had a normal value at baseline and an abnormal value within 12 hours of discontinuation of I-NO.

b. These subjects include all of those in the 'new abnormalities' category, as well as any subject who had an abnormal value at baseline which was more abnormal on the follow-up lab.

c. Data was obtained from NDA volume 2.31, Data Listing 13.1; volume 2.25, Appendix 16.2.2.12; and volume 2.18, Table T-30, and electronic datasets.

Another source of data on the effects of I-NO on LFTs is the individual subject lab data. The subjects who experienced a markedly abnormal AST from the individual labs was identified (from Table 8.1.6.2.2.1a.1). While no control subject was identified, 4 I-NO subjects (3%) were identified. No follow-up labs are available for the subjects listed.

Table 6.6.14 (from 8.2.2.2.5) Individuals with markedly abnormal AST post-I-NO from INO-01/ -02 and /-03[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo	None			
I-NO 5 ppm				
02-11008	SGOT	78	145	Discharged without ECMO with CLD[c]
I-NO 20 ppm				
01-03025	SGOT	109	358	Died
01-03008	SGOT	181	264	Discharged without ECMO with seizures
I-NO 80 ppm				
01-02003	SGOT	69	120	Discharged without ECMO data missing

a. Data from NDA, volume 2.25, individual patient listings, and from electronic datasets.

b. Lab tests were identified as markedly abnormal were >2X upper limits of normal on post-I-NO value. Normal values were taken from individual lab ranges associated with each specimen.

c. CLD: chronic lung disease.

6.6 Laboratory Abnormalities (cont)

Individuals in all treatment groups also had marked elevations in their LDH and/or bilirubin. No markedly abnormal alkaline phosphatase values were identified.

Table 6.6.15 (from 8.2.2.2.6) Individuals with markedly abnormal LDH and total bilirubin post-I-NO chemistry labs from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value
Placebo			
01-03013	LDH	550	720
01-04001	LDH	515	527
02-14004	Bilirubin	9.3	14.3
01-14002	Bilirubin	9	17.2
01-07007	Bilirubin	10.5	15
I-NO 5 ppm			
01-03002	LDH	517	939
01-06002	LDH	1955	3981
02-14001	LDH	530	1235
02-15001	LDH	510	1022
01-01004	Bilirubin	5.5	13.7
I-NO 20 ppm			
01-17006	LDH	2939	3946
01-07003	Bilirubin	5.9	14.2
01-07005	Bilirubin	12.9	13.9
01-09003	Bilirubin	18.9	30.1
01-14001	Bilirubin	9.5	14.1
03-52001	Bilirubin	11	15.3
I-NO 80 ppm			
01-03003	LDH	475	692
01-06003	LDH	1936	2995
01-11004	LDH	3429	6270
02-04004	LDH	508	1098
01-02003	LDH	763	1623
02-11007	LDH	1534	2517
03-59003	LDH	NA	1634
01-05003	Bilirubin	10.9	13.4
01-03005	Bilirubin	9.8	13.5
02-13001	Bilirubin	9.4	13.1

a. Data from NDA, volume 2.25, individual patient listings, and electronic datasets.
b. Lab tests were identified as markedly abnormal were >2X upper limits of normal on post-I-NO value.

Conclusion

Given the large fraction of infants with abnormal elevations in one or more of these at baseline, and the small number of infant with available data, we are limited in our power to detect such an effect of I-NO. Overall, however, no clear association between I-NO administration and abnormalities in AST, Alk Phos, and Total Bilirubin were detected. No chronic damage, and no deaths due to hepatic failure were identified in the database.

6.7 Other Laboratory Measurements

The other laboratory measurements performed in the INO-01/ -02 trial are reviewed in the previous document dated 11.19.97. A review of the laboratories measured in the CINRGI trial reveal that, with the exception of the labs discussed above, no relevant differences between the two treatment groups is suggested by the data.

6.8.Miscellaneous Adverse Events

In this section adverse events that are normally evaluated as part of an NDA review are summarized.

GI Bleeding

The incidence of GI bleeds through the time of discharge was collected in the NINOS trials.

Table 6.8.1 (from 6.0.1.13.1.2) Comparison of specific safety parameters during the NINOS trial*.

Characteristic	Placebo Group (n=121)	I-NO Group (n=114)	p value*
GI bleeding	1/109 (1%)	1/107 (1%)	0.99

Intracranial Bleeding

The incidence of intracranial bleeding was examined in the NINOS, INO-01/ -02 and CINRGI trials, and the results are summarized in the Acute Neurologic Injury Section above (section 6.4). Overall, no difference in the rate of these bleeds, both detected clinically and those detected through radiological procedures (U/S, CT scans) was detected between the control and I-NO groups in any of the trials.

Allergic Reactions

Part of a normal safety review includes a discussion of the allergic potential of the product. No additional information has become available since my original review, where I concluded that the data are insufficient to assess I-NO for the potential to cause an allergic reaction.

Tolerance

The reader is referred to the discussion of tolerance in section 8.1.9.1 of my original review. I concluded that the best available evidence from INO-01/ -02 suggests that tolerance does not develop to a significant degree in that trial population, the literature has raised the possibility of it in isolated patients. Animal models have not been used to address this issue to the knowledge of this reviewer.

Rebound Hypoxia and Pulmonary Hypertension

This refers to the abrupt increase in pulmonary artery pressures or decline in oxygenation following discontinuation of I-NO. In section 8.1.9.2 of my original review, I concluded that there is evidence for rebound hypoxia following abrupt discontinuation of I-NO, a conclusion shared by the sponsor. The evidence for rebound pulmonary hypertension is also reviewed in section 8.1.9.2, and is limited to published literature, as no such measurements were made in any of the trials in the NDA.

Abuse Potential

No data exists on the abuse potential of I-NO to the knowledge of this reviewer, but its potential for abuse is expected to be low. Nitrous oxide, N_2O, is not related to this compound, and is not formed during the use of I-NO as proposed in the NDA.

Overdose

Human overdose data is quite limited with regards to I-NO, although two subjects in the NINOS trial were given 100 ppm I-NO inadvertently.

1. Patient #54-A02: this 3.7 kg female received I-NO for PPHN with meconium aspiration syndrome, with a baseline OI of 23. After responding to 20 ppm I-NO, she was continued on study gas for 159 hours (day 5), at which time her dose was inadvertently increased to 100 ppm for approximately 36 minutes. While no coincident methemoglobin and NO_2 levels were obtained, her higher recorded levels were 2.2% methemoglobin on day 3 and 0.4 ppm NO_2 on day 1. She was weaned with difficulty, did not receive ECMO, and was discharged, with no chronic lung disease or excess bleeding or other major organ dysfunctions.

2. Patient #3-A02: this 2.9 kg female received I-NO for PPHN with pneumonia/sepsis, with a baseline OI of 56 and 26. She had a partial response to I-NO 20 ppm, and no response to I-NO 80 ppm. An improper flow-meter setting led to her exposure to I-NO 101 ppm for approximately 1 hour. Her methemoglobin level at that time was 6% and her NO_2 level was 5.1 ppm. Study gas was weaned down to 20 ppm and the NO_2 fell to 3.4 ppm. After 14 hours more, she received ECMO for persistently elevated A-aDO_2. She survived, but was discharged to home on O_2.

One subject in the NINOS trail received I-NO for more than 240 hours.

3. Subject #51-A12: this female received I-NO for PPHN with meconium aspiration. She had a partial response to I-NO 20 ppm. At 240 hours, the infant was on 20 ppm, and was ultimately weaned after a total of 253 hours and 25 minutes. Her maximum NO_2 and methemoglobin levels were 0.1 and 3.6 respectively. The subject received high-frequency ventilation while receiving study gas, and developed both pneumothorax and periventricular leukomalacia. Chronic lung disease was also diagnosed, after the infant required ventilation for 16 days.

7.0 Overall Summary of Efficacy and Safety for I-NO

The data from the three pivotal trials (NINOS, INO-01/ -02 and CINRGI) support the contention that I-NO administration is associated with a significant decrease in the use of ECMO. In two of these trials, this result was part of a pre-specified primary endpoint (NINOS, CINRGI). This effect of I-NO to decrease ECMO use may well be due to the acute effect of I-NO to improve oxygenation, rather than due to any other beneficial effect on the course of the disease causing the hypoxic respiratory failure. In this interpretation, the acute effect of I-NO to improve oxygenation leads to a conclusion by the physician that the child is 'improving' because he or she is 'pinker.' As a result of this conclusion, the physician is less likely to initiate ECMO.

In support of this contention, no beneficial effect of I-NO on mortality or any other clinical endpoint was demonstrated, or strongly suggested, by the combined data. In the larger context, mortality rates for PPHN have been falling significantly from approximately 50-60% in the 70s and 80s, to approximately 15-20% today. This has been accomplished without the use of I-NO, suggesting that other therapies (HFOV/HFJV, surfactant, alkalization, improved ICU monitoring) are having a positive impact on neonatal hypoxic respiratory failure. The falling mortality rate also mitigates against the argument that no effective therapy for PPHN now exists, so that a less than overwhelmingly effective drug might still require approval. This also means that the safety of I-NO needs to be well-defined prior to wide-spread use.

The following safety issues have been raised during one or both of my reviews:

1) The safety database included small number of subjects, and for most adverse events, the INO-01/ -02 was the primary source of information. Given the baseline differences between the subjects in the INO-01/ -02 and the other trials, extrapolating between the two populations is also difficult, and open to serious errors of omission due to inadequate data. These difficulties have been alleviated to some extent by the addition of 97 additional patients who were exposed to I-NO, bringing the total number of children exposed to I-NO in the NDA database to 375. The difficulties with differences in baseline characteristics are again present in the CINRGI trial, complicating its interpretation. Another potentially confounding variable between the CINRGI trial and the previous trials is the lower dose of I-NO administered in CINRGI (20 ppm reduced to 5 ppm if possible), compared with the NINOS and INO-01/ -02 trials (20-80 ppm).

2) The available safety database in the original NDA raised several potential safety issues. The most troubling of the adverse events, raised in the original medical review, was the possible association of I-NO with acute and chronic pulmonary toxicities. This association, like all of the safety data, relied on small numbers of subjects, although the association was plausible, given the available data. The addition of the CINRGI trial data, along with additional long-term follow-up data from NINOS and INO-01/ -02 has allayed some of the concerns, especially regarding the occurrence of chronic injury. The existing database is inadequate, however, to exclude the occurrence of pulmonary toxicity in association with the use of I-NO.

3) There was a definite association of I-NO with the development of methemoglobinemia and elevated NO_2 concentrations, identified in the NINOS and INO-01/ -02 trials (especially at the 80 ppm dose). This concern is minimized with the use of the lower doses of I-NO in the CINRGI trial (and the proposed dose for the label).

4) Several other adverse events were also possibly linked to the administration of I-NO based on the data available in 1997, although the data were insufficient to determine the seriousness of these potential adverse events, or to determine their duration or dose-response. The addition of the CINRGI data has resolved some of these safety concerns, and no new safety concerns have arisen as a result of the CINRGI trial review. The available data does suggest that rapid discontinuation of I-NO is associated with rebound hypoxia in some patients.

5) For some adverse events of interest, no data were obtained at all. Most critical of these was the effect of I-NO on coagulation parameters. Other clinical events for which we have either scarce or no clinical data include: musculoskeletal injury; non-glomerular renal injury; effects on the cardiac conduction system, and effects on serum electrolytes.

6) The number of patients exposed to I-NO is too small to adequately assess the potential interactions of I-NO with disease states, patient demographics and concomitant medications. The potential interaction of I-NO with other drugs is of particular importance for drugs commonly used to treat this condition, such as steroids and vasodilators (with the exception of tolazoline).

7) Finally, an issue that cannot be resolved from the database is the potential genotoxicity and carcinogenicity of I-NO. The available data on the genotoxicity of I-NO are mixed (see section 4.1 in my 1997 review for details). It is true that the duration of exposure to I-NO is limited in these studies, and that I-NO is produced (at many-fold lower concentrations) intracellularly. However, the cumulative years of risk for a newborn who receives I-NO is appreciably longer than an adult.

7.0 Overall Summary of Efficacy and Safety for I-NO (cont)

With regard to the efficacy of I-NO, one or both of my reviews also raised the following points:

1) The variable response to I-NO in some of the subsets of infants, particularly in relation to their underlying disease state, suggests that the effect of I-NO may not been equivalent across all infants with hypoxic respiratory failure.

2) The limited number of patients with congenital diaphragmatic hernia in CINRGI is too small to make firm conclusions about I-NO efficacy and safety in this sub-group, but no trend toward efficacy was seen.

3) No effect of I-NO on mortality (beneficial or adverse) has been demonstrated by the available data.

The data, then, suggest that I-NO has a dose-dependent, acute effect on oxygenation in newborns with hypoxic respiratory failure which translates into a perception on the part of the clinician taking care of the infant of clinical improvement, leading to a lower rate of use of ECMO. There are no data demonstrating a clear beneficial effect of I-NO on hard-endpoints (death, days of hospitalization, days of ventilation, incidence of chronic lung disease or neurological sequelae), in an era with other effective therapies and falling mortality rate.

Two things have changed since the initial submission that are most relevant to the approval decision for I-NO. First, the CINRGI trial is a second trial demonstrating the significant reduction in ECMO in these patients. Second, in distinction to the situation in 1997, the available database is more reassuring regarding the potentially serious safety concerns related to I-NO, especially the potential for lung injury, for the 20 ppm dose of I-NO. The safety database is inadequate as regards to certain key adverse events, and the possible interactions of I-NO with other vasoactive substances are yet to be firmly established.

These data allow the following conclusion. While a clinical benefit of I-NO in this population as we usually define it has not been demonstrated, a beneficial effect of I-NO on the physiology of these severely ill patients (durable improvement in oxygenation) has been demonstrated. This improvement in physiology translates into a reduction in the use of ECMO, an invasive procedure with important morbidity and mortality risks. While the database does not address some safety concerns for I-NO, the available data suggest that the short-term use of I-NO is not associated with severe toxicity. While the concerns about the potential pulmonary toxicity have been alleviated by the additional data, additional data are needed to exclude an adverse pulmonary effect of I-NO.

The question is whether the improvement of oxygenation by I-NO, combined with the evidence of decreased use of ECMO from two trials, is a sufficient benefit to warrant approval. In October of 1995, the Cardiovascular and Renal Drugs Advisory Committee met to discuss investigation of I-NO in pediatric respiratory failure. The committee ultimately voted against the use of oxygenation parameters (in particular the OI) as a valid efficacy endpoint for I-NO trials in neonatal respiratory failure. The result was 14 votes against the use of improvement in oxygenation, especially changes in OI, as a primary efficacy endpoint, with 4 supportive votes, and 5 abstentions.

It is also worth looking at the other drugs that alter 'physiologic' or laboratory measurements. Of the drugs that have been given approval for altering laboratory measurements (e.g., potassium supplement, phosphate binders, drugs to lower cholesterol) one class appears to rely on a similar imputed benefit of an acute change in a serum chemistry without any relationship to long-term benefit: drugs used to treat acute hypercalcemia.

In some important ways, progressive hypercalcemia mimic those of progressive hypoxia with regard to clinical course and in the use to avoid a more invasive medical procedure. Both hypoxia and hypercalcemia lead to altered mental status. In hypercalcemia, while there is a continuum of progressively higher Ca^{2+} levels, in which the clinician feels progressively more unease if he or she does not intervene, there is no single level above which there is recognized uniform clinical morbidity. A similar course is seen in hypoxia. At some point both are fatal in all patients, but that point is not uniform. Finally, just like the avoidance of ECMO with I-NO, the treatment of hypercalcemia with drugs can be seen as averting a more invasive/dangerous procedure (hemodialysis or peritoneal dialysis). If this analogy is accepted, the approved treatments of hypercalcemia and I-NO would both be as useful tools to use in the short-term to halt a pathologic change (rising Ca^{2+}, falling O_2). Their effect, then, is not necessarily alter the disease process; it is rather to allow other therapies to have clinical effect. For hypercalcemia in malignancies, this might by chemotherapy. In hypoxic respiratory failure of the newborn, this might be surfactant or antibiotics. The effect is the same: no clinical benefit can be shown by I-NO or pamidronate (as an example), but failure to use them can have universally-recognized adverse outcomes (death, ECMO or Hemodialysis).

7.0 Overall Summary of Efficacy and Safety for I-NO (cont)

The analogy (like most analogies) is not perfect, as drugs for the treatment of hypercalcemia also have additional indications (i.e., treatment of Paget's disease and post-menopausal osteoporosis). The indication for hypercalcemia does, however, yield a possible model for I-NO approval. If this reasoning is insufficient, and we require demonstration of a clear clinical benefit, than the current NDA database fails to support the approval of I-NO in this population, as the use of ECMO is too tightly linked to the changes in oxygenation to suffice as a stand-alone clinical benefit.

Approval of I-NO in this way requires that I-NO also have a clearly defined safety margin. A clearly-defined safety margin is even more critical in this case, as the population to be served are critically-ill neonates. In the case of I-NO, the newly-available data suggest that it does, indeed, have a broader margin for safe use at the 20 ppm dose. As discussed above, while there are some adverse effects for which no information was collected in the database, and others for which insufficient data exist, no adverse event has been identified that is clearly linked to I-NO association except for the development of methemoglobinemia and increased NO_2 concentrations. One are of persistent concern, that the data are insufficient to answer, is the possible link of I-NO to acute lung injury, particularly as reflected in the increased number of pneumothoraces in the INO-01/ -02 trial. Additional data are needed to determine whether this adverse effect is indeed linked to I-NO.

In balance, then, I conclude that I-NO is approvable for the short-term treatment of hypoxia associated with respiratory failure in newborns.

8.0 References

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9.0 Appendix One: list of abbreviations used in the review

a/AO_2: arterial-alveolar oxygen ratio

$A\text{-}aDO_2$: alveolar-arterial oxygen tension gradient in torr. Calculated as $A\text{-}aDO_2 = P_AO_2 - P_aO_2$, where alveolar partial pressure of oxygen = $FiO_2 \times 723$ mmHg - $(paCO_2/0.8)$.

ALS: Air Leak Syndrome. Occurrence of any of the following: pneumothorax; pneumomediastinum; pneumopericardium; pneumoperitoneum; subcutaneous or interstitial emphysema.

ECMO: extracorporeal membrane oxygenation

FIO_2: fractional of inspired O_2 expressed in % of total inspired gases.

HFJV: high-frequency jet ventilation

HFOV: high-frequency oscillatory ventilation

MAS: meconium aspiration syndrome, defined as the presence of two of the following three findings:

1. History of meconium at delivery.
2. Suctioning of meconium from the endotracheal tube.
3. Radiographic evidence of MAS.

MetHb: Methemoglobin (in %).

mmHg: pressure in millimeters of mercury.

OI: Oxygenation index, calculated at $(PAW \times FiO_2)/ PaO_2$ in cm H_2O/mmHg.

P_AW: mean airway pressure.

PEEP: positive end-expiratory pressure

PIP: positive inspiratory pressure

PPHN: Persistent pulmonary hypertension of the newborn. It is defined in NINOS as:

1. No echocardiographic evidence of structural heart disease.
2. No radiographic evidence of parenchymal pulmonary disease.
3. One or more of the following indications of elevated pulmonary artery pressure:
    a. Patent ductus arteriosus with right-to-left or bi-directional shunt.
    b. Patent foramen ovale with right-to-left or bidirectional shunt.
    c. Tricuspid regurgitation with elevated pulmonary artery pressure.
    d. Elevated systolic time interval >0.3 seconds.
    e. Contrast echocardiography evidence of right-to-left shunt.
    f. Color flow confirmation of delayed pulmonary valve closure using M-mode Doppler.
    g. Echocardiographic evidence of dilated left ventricle.
    h. Echocardiographic evidence of flattened ventricular septum.

Post-Ductal $O_2/TCPPO_2$ (%): O_2 saturation post-patent ductus arteriosus in %

ppm: parts per million, by volume (80 ppm = 0.008% of the inhaled gas)

Pre-Ductal $O_2/TCPPO_2$ (%): O_2 saturation pre-patent ductus arteriosus in %

RDS: Respiratory Distress Syndrome, defined in NINOS as the presence of both of the following:

1. Gestational age <37 weeks.
2. Findings on chest x-ray consistent with RDS or a lecithin/sphingomyelin ratio <2.

Sepsis/Pneumonia: diagnosis made in NINOS with the presence of at least 1 of the following:

1. Positive blood culture.
2. Positive cerebrospinal fluid culture or gram stain.
3. Findings on CXR consistent with pneumonia.
4. Clinical or CXR findings consistent with pneumonia or

Clinically unstable neonate with at least two major criteria of suspected sepsis(unexplained hypotension; unexplained lethargy; unexplained apnea or respiratory distress; gray skin or sclera; leukopenia (WBC count <5000 cells/µl or >10% band forms)' thrombocytopenia) or at least two minor criteria (maternal amnionitis; maternal fever before delivery; fetal tachycardia >160 beats per minutes; prolonged rupture of membrane >24 hours).

Time-weighted OI: the area under the OI/time curve either through 24 hours or until discontinuation, divided by the number of hours of exposure to the treatment gas.

10.0 Appendix Two: Narratives of Deaths

Deaths in the Placebo Group

1. Patient akrcdh2: The patient was a 3.0 kg Hispanic female product of a 39 week gestation, born by vaginal delivery on 12/14/98 to a 25 year old gravida 3, para 2 mother with treated group B streptococcus and late prenatal care. The patient had Apgar scores of 4 (1 minute), 6 (5 minutes) and 6 (10 minutes). She was diagnosed with a left congenital diaphragmatic hernia and a right pneumothorax. She rapidly developed signs of persistent pulmonary hypertension of the newborn. She was started on placebo gas at 5 hours of life. She met treatment failure criteria within 3 hours of the initiation of study gas. ECMO was begun at 10 hours of life and was successfully discontinued on 12/17/98. She underwent a repair of her diaphragmatic hernia on 12/24/98, however her persistent pulmonary hypertension worsened post-operatively. On 12/31/98 a decision was made to withdraw her life support and she died shortly later.

2. Patient appphn8: The patient was a 3.1 kg white female product of a 33 week gestation, born by emergent C-section on 5/26/98 to a 35 year old gravida 3, para 2 mother, whose pregnancy was complicated by polyhydraminos requiring amnioreduction. The patient had Apgar scores of 1 (1 minute) and 4 (5 minutes). She was diagnosed as having persistent pulmonary hypertension of the newborn associated with her history of fetal cystic hygroma and severe hydrops. She was started on placebo gas at 13 hours of life. She failed to respond to treatment and was declared a treatment failure at 24 hours. The patient's status continued to deteriorate and on 6/2/98 a decision was made to withdraw life support.

3. Patient chcdh1: The patient was a 2.3 kg white female product of a 37 week gestation, born by vaginal delivery on 8/18/97 to a 38 year old mother. The patient had Apgar scores of 6 (1 minute) and 8 (5 minutes). She had a right congenital diaphragmatic hernia and rapidly developed signs of persistent pulmonary hypertension of the newborn. At 9 hours of life, placebo gas was begun. The patient had no improvement and the gas was withdrawn at 13 hours of life as part of the decision to withdraw life support. The patient expired a short time later.

4. Patient cmccdh3: The patient was a 3.4 kg white male product of a 40 week gestation, born by elective C-section on 4/8/97 to a 30 year old gravida 2, para 2 mother. The patient had Apgar scores of 1 (1 minute) and 3 (5 minutes). He was noted to have a left congenital diaphragmatic hernia and rapidly developed signs of persistent pulmonary hypertension of the newborn. He was placed on placebo gas on 4/10/97 but within 4 hours met treatment failure criteria for "failure to respond". During the ECMO cannulation procedure, the patient suffered a cardiac arrest which responded to resuscitation. The patient was found to have a large pericardial effusion, and open heart surgery was performed to close the small perforation in the right atrium. On 4/12/97, the patient developed a grade III intraventricular hemorrhage and began to have seizures. Because of the infant's deterioration, a decision was made to withdraw life support on 4/12/97.

5. Patient cmcpphn2: The patient was a 1.9 kg white male product of a 37 week gestation, born by emergent C-section on 5/17/96 to a 24 year old gravida 1, para 0 mother. The patient had Apgar scores of 1 (1 minute) and 3 (5 minutes). He was diagnosed as having sepsis and persistent pulmonary hypertension of the newborn. On 5/18/96, the patient was noted to have a seizure, and, on the morning of 5/19/96, he developed bilateral pneumothoraces. He was placed on placebo gas on 5/19/96. Within 30 minutes, he met the treatment failure criteria of "failure to respond" and "need for ECMO". He was placed on ECMO until 5/27/96 when he was discovered to have a large bilateral grade III intraventricular hemorrhage and developed seizures. A decision was made to withdraw life support on 5/31/96.

6. Patient echpphn9: The patient was a 4.3 kg white female product of a 40 week gestation, born by non-emergent C-section for breech presentation on 7/14/97 to a 29 year old gravida 7, para 0 mother, whose pregnancy was complicated by insulin dependent diabetes, obesity, hypertension and multiple prior spontaneous abortions. The patient had Apgar scores of 0 (1 minute) and 2 (5 minutes). She was diagnosed as having persistent pulmonary hypertension of the newborn. She was started on placebo gas at 12 hours of life. She met the treatment failure criteria of "failure to respond" and "urgent need for ECMO" after 4½ hours of gas treatment. She developed parenchymal bleeding bilaterally and acute renal failure. Her condition continued to deteriorate, and the decision was made to withdraw life support on 7/23/97.

Deaths in the Placebo Group (cont)

7. Patient musccdh3: The patient was the 2.5 kg black male product of a 38 week gestation, born by vaginal delivery on 9/18/98 to a 31 year old gravida 3, para 1 mother, whose pregnancy was complicated by intrauterine growth retardation. The patient had Apgar scores of 2 (1 minute) and 5 (5 minutes). He was noted to have a left congenital diaphragmatic hernia and rapidly developed signs of persistent pulmonary hypertension of the newborn. He was started on placebo gas on 9/19/98. The gas was discontinued within 21 hours when the patient met ECMO criteria. After the initiation of ECMO, the patient was noted to have cardiac stun with poor cardiac flow. While on ECMO, the patient developed right upper lobe atelectasis and a pericardial effusion. ECMO was successfully discontinued on 10/7/98. He underwent surgical repair of his hernia on 10/9/98, and, on 10/18/98, he was noted to have cardiac hypertrophy with poor filling. On 10/21/98, the patient developed a persistent acidosis and significant hypoxemia. He experienced severe bradycardia which did not respond to resuscitation efforts and he expired.

8. Patient muscpn12: The patient was the 3.8 kg black female product of a 40 week gestation, born by vaginal delivery on 6/13/98 to a 27 year old mother. The patient had Apgar scores of 7 (1 minute) and 8 (5 minutes). She had Down's Syndrome associated with congenital heart disease consisting of a ventricular septal defect overriding her aorta with bidirectional flow, a large atrial septal defect, a patent ductus arteriosus, and hypertrophy of the right ventricle. She developed persistent pulmonary hypertension of the newborn secondary to pneumonia. Placebo gas was initiated at 14 hours of life. She met the treatment failure criteria of "urgent need for ECMO" within 2 hours. ECMO was started and continued through 6/17/98. While on ECMO, she developed a right pneumothorax which resolved. Her respiratory status deteriorated and she was started on compassionate use inhaled nitric oxide. Her condition continued to deteriorate and a decision was made to withdraw life support on 6/26/98.

9. Patient muscpn5: The patient was the 3.1 kg white male product of a 37 week gestation, born by vaginal delivery on 1/10/97 to a 33 year old gravida 2, para 1 mother whose pregnancy was complicated by prolonged rupture of membranes for 20 hours prior to delivery and maternal fever of 100° Fahrenheit. The patient had Apgar scores of 7 (1 minute) and 9 (5 minutes). He was diagnosed as having persistent pulmonary hypertension of the newborn which was felt to be the result of a group B streptococcal pneumonia. He also developed evidence of septic shock. On 1/11/97, he was placed on placebo treatment gas. The patient met the treatment failure criteria of: "failure to respond", "condition worsened", and "an urgent need for ECMO" within 5 minutes of initiation of treatment gas and was cannulated for ECMO. During the ECMO procedure, the patient suffered a cardiac arrest from which he was successfully resuscitated after a period of 15 minutes. While on ECMO, he developed a massive intracranial hemorrhage on the left side with extension into the brain parenchyma. A decision was made to withdraw life support on 1/12/97.

10. Patient occdh1: The patient was a 2.0 kg white male product of a 36 week twin gestation, born by non-emergent C-section on 8/26/97 to a 26 year old mother whose pregnancy was complicated by gestational diabetes, preterm labor and "non-reassuring" fetal heart tones. The patient had Apgar scores of 0 (1 minute) and 3 (5 minutes). He was noted to have a right congenital diaphragmatic hernia and rapidly developed signs of persistent pulmonary hypertension of the newborn. He was started on placebo gas at 11 hours of life. Within 3 hours he met treatment failure criteria of "failure to respond" and "met ECMO criteria". On 9/11/97, the patient underwent surgical repair of his congenital diaphragmatic hernia, and on 9/14/97 ECMO treatment was discontinued. His hospital course was complicated by diffuse tracheobronchial malacia, respiratory syncytial viral pneumonia and bronchiolitis. He received "compassionate use" inhaled nitric oxide because of documented pulmonary artery hypertension. His respiratory status continued to deteriorate, and he became unresponsive to medical interventions. On 1/12/98, he developed progressive bradycardia which progressed to asystole despite resuscitative measures.

Deaths in the Placebo Group (cont)

11. Patient sjhcdh2: The patient was a 2.9 kg Hispanic male product of a 38 week gestation, born by vaginal delivery on 6/12/98 to a 27 year old gravida 3, para 2 mother whose pregnancy was complicated by polyhydraminos which was treated by amnio-reduction. The patient had Apgar scores of 3 (1 minute) and 3 (5 minutes). He was noted to have a left congenital diaphragmatic hernia and pulmonary hypoplasia. He rapidly developed signs of persistent pulmonary hypertension of the newborn. He was started on placebo gas on 6/15/98 and remained on gas for the maximum duration of treatment allowed in the trial of 96 hours. On 7/4/98, he was placed on ECMO for his persistent pulmonary hypertension of the newborn. While on ECMO, he developed renal failure requiring hemodialysis. ECMO was discontinued after 262 hours of treatment on 7/15/98. He was then placed on continuous renal replacement therapy and hemodialysis. His hospital course was complicated by the development of significant pulmonary hemorrhage, E. coli sepsis, significant cholestasis associated with an enlarged and thickened gall bladder and left flank cellulitis. Upon redeveloping severe pulmonary hemorrhage and subsequently thrombosis of the dialysis catheters, a decision was made to withdraw life support on 7/22/98.

Deaths in the Inhaled NO Group

1. Patient apcdh2: The patient was a 2.7 kg white female product of a 39 week gestation, born by vaginal delivery on 6/21/97 to an 18 year old gravida 2, para 0 mother whose pregnancy was complicated by polyhydraminos and hypertension. The patient had Apgar scores of 1 (1 minute) and 5 (5 minutes). She was diagnosed has having pulmonary hypoplasia secondary to the polyhydraminos and persistent pulmonary hypertension of the newborn. At 9 hours of life, she was started on inhaled NO. She met the treatment failure criterion of "need for ECMO" after 2 hours of treatment. She received ECMO therapy until 7/5/97 when she developed both right and left pleural effusions. Her respiratory status continued to deteriorate and she expired on 7/20/97.

2. Patient chcdh2: The patient was the 3.3 kg Hispanic male product of a 39 week gestation, born by vaginal delivery on 10/29/97 to a 27 year old gravida 3, para 1 mother. The patient had Apgar scores of 7 (1 minute) and 8 (5 minutes). He was noted to have a congenital diaphragmatic hernia and rapidly developed persistent pulmonary hypertension of the newborn. He was started on inhaled NO on 10/30/97. Within the hour, he went the treatment failure criteria of "failure to respond" and "the need for ECMO". ECMO was started and continued through 11/20/97. His hospital course was complicated by the development of gastrointestinal bleeding, disseminated intravascular coagulopathy, and renal failure requiring hemodialysis. A decision was made to withdraw life support on 12/28/97.

3. Patient cmccdh1: The patient was a 3.2 kg white female product of a 40 week gestation, born by vaginal delivery on 7/19/96 to a 33 year old gravida 1, para 0 mother. The patient had Apgar scores of 1 (1 minute) and 4 (5 minutes). She was noted to have a left congenital diaphragmatic hernia and rapidly developed signs of persistent pulmonary hypertension of the newborn. She was started on inhaled NO at 7 hours of life. Within 30 minutes, she met the treatment failure criteria of "failure to respond" and "need for ECMO". ECMO was started and continued through 7/29/96. She did poorly and ECMO was reinitiated on 8/3/96. On 8/8/96, she underwent surgical repair of her left congenital diaphragmatic hernia during which a large right pleural effusion was noted. Her condition continued to deteriorate, and on 8/12/97, a decision was made to discontinue life support.

4. Patient cmcpn1: The patient was a 3.8 kg white male product of a 40 week gestation, born by vaginal delivery on 11/30/97 to a 17 year old gravida 1 para 0 mother. The patient had Apgar scores of 5 (1 minute) and 9 (5 minutes). He was diagnosed as having septic shock and associated persistent pulmonary hypertension of the newborn. He was started on inhaled NO at 19 hours of age. Within 8 hours, he met the treatment failure criteria of "failure to respond" and "need for ECMO". While on ECMO, he developed kidney, liver and central nervous system dysfunction associated with seizure activity. The patient's condition continued to deteriorate and on 12/11/97 the decision was made to discontinue his life support.

Patient echrds2: The patient was a 3.4 kg black male product of a 42 week gestation, born by vaginal delivery on 5/2/96 to a 33 year old gravida 4, para 2 mother. The patient had Apgar scores of 5 (1 minute) and 9 (5 minutes). He was diagnosed as having respiratory distress syndrome and persistent pulmonary hypertension of the newborn. He was placed on high frequency oscillatory ventilation and inhaled NO on 5/3/96. Inhaled NO was stopped the following day after significant improvement.

Deaths in the I-NO Group (cont)

4. (cont) The patient remained HFOV dependent and on 5/20/96 a lung biopsy was performed which revealed that he had obliterative bronchiolitis from adenovirus. Despite aggressive therapy he continued to deteriorate and, on 5/27/96, a decision was made to discontinue life support.

5. Patient echrds5: The patient was a 2.6 kg Hispanic male product of a 40 week gestation, born by vaginal delivery on 11/14/96 to a 21 year old gravida 1, para 0 mother whose pregnancy was complicated by treated group B streptococcal culture. The patient had Apgar scores of 7 (1 minute) and 7 (5 minutes). He was diagnosed as having respiratory distress syndrome and group B streptococcal sepsis with associated persistent pulmonary hypertension of the newborn. He was placed on inhaled NO on 11/16/96 and remained on treatment gas for the maximum study gas duration of 96 hours. He developed a pneumothorax. He continued to deteriorate and on 12/2/96, a decision was made to withdraw life support.

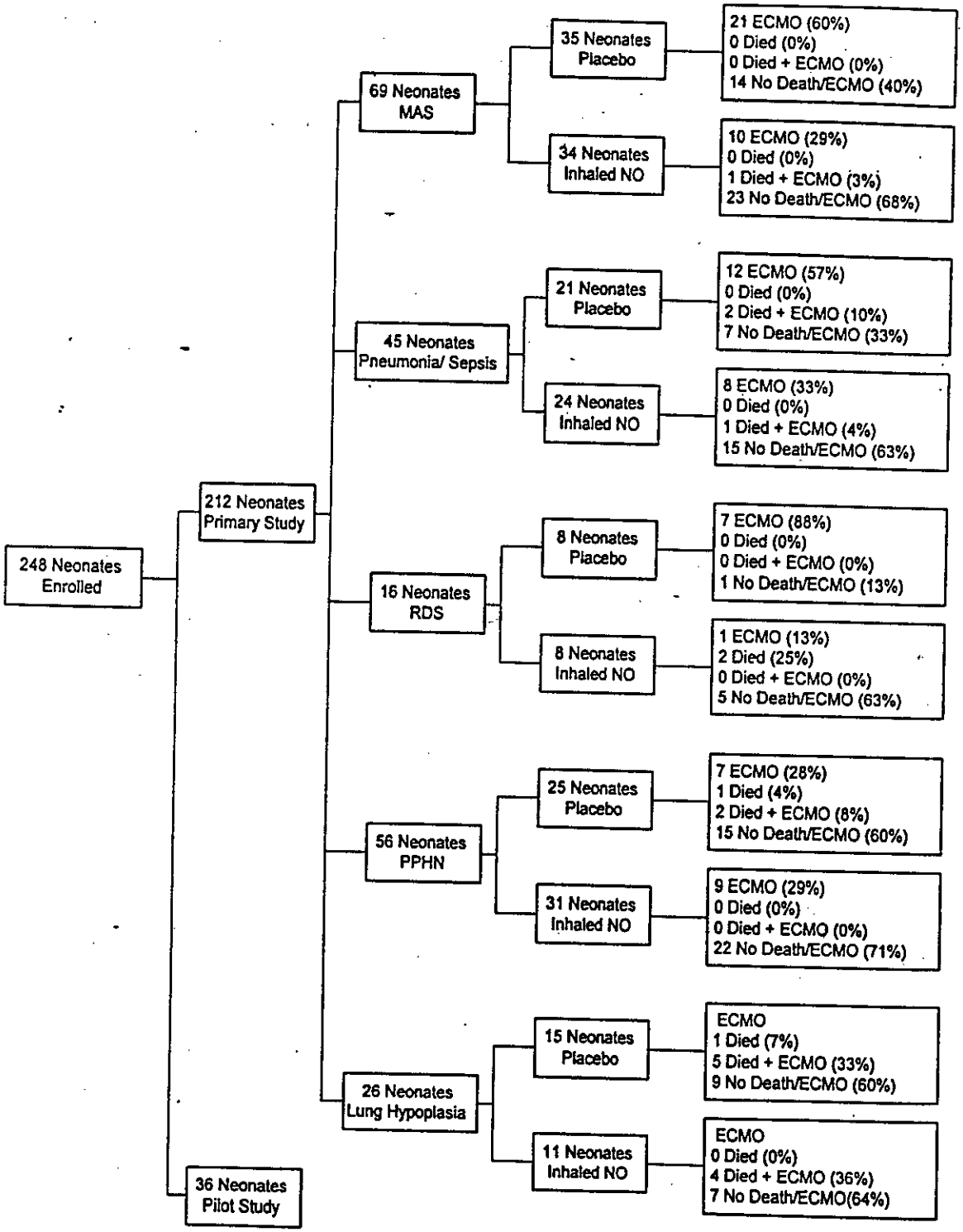
6. Patient guhmas2: The patient was the 3.2 kg Asian female product of a 40 week gestation, born by vaginal delivery on 10/28/96 to a 30 year old gravida 3, para 0 mother with gestational diabetes controlled by diet. The patient had Apgar scores of 6 (1 minute) and 8 (5 minutes). She developed persistent pulmonary hypertension of the newborn believed to be the result secondary to clear amniotic fluid aspiration. She was placed on inhaled NO on 10/29/96. She met the treatment failure criterion of "urgent need for ECMO" within a few minutes. ECMO was started at 10 hours of life. While on ECMO, the patient developed a right pneumothorax and copious bleeding into the chest tube. She was transferred to another hospital on 11/19/96 while still on ECMO. She failed to improve and a decision to discontinue life support was made on 12/8/96.

7. Patient musccdh1: The patient was the 3.6 kg white male product of a 38 week gestation, born by vaginal delivery on 5/19/98 to a 26 year old gravida 2, para 1 mother. The patient had Apgar scores of 5 (1 minute) and 6 (5 minutes). He was noted to have a left congenital diaphragmatic hernia and he developed signs of persistent pulmonary hypertension of the newborn. He was started on inhaled NO at 9 hours of life. He met the treatment failure criterion of "need for ECMO" after 13 hours. The patient was placed on ECMO on 5/20/98 and continued on ECMO until 6/4/98. On 6/5/98, the patient underwent surgical repair of his left congenital diaphragmatic hernia. The patient's condition worsened and he developed renal failure. A decision was made to withdraw life support on 6/16/98.

11.0 Appendix Three: Patient Disposition in CINRGI

APPEARS THIS WAY
ON ORIGINAL

FIGURE 1
DISPOSITION OF PATIENTS



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Proposed Label

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cc: ORIG: NDA 20-845
HFD-110 Division File

HFD-110/CSO	Zelda McDonald
HFD-110/Medical Officer	Douglas Throckmorton
HFD-110/Secondary Reviewer	Norman Stockbridge

McDonald

NOV 24 1997

INTEGRATED MEDICAL REVIEW OF NEW DRUG APPLICATION

NDA: 20-845
DRUG NAME: I-NOmax™ (nitric oxide for inhalation)
TRADE NAME: N/A
FORMULATION: Gas for inhalation
SPONSOR: Ohmeda Pharmaceuticals Products Division, Inc.
TYPE OF DOCUMENT: New Drug Application

MEDICAL REVIEWER: Douglas C. Throckmorton, M.D.

PROPOSED INDICATIONS: Treatment of term and near-term infants with hypoxic respiratory failure.

DATE OF NDA SUBMISSION: 6.16.97
DATE RECEIVED BY FDA: 6.17.97
DATE ASSIGNED: 6.17.97
DATE REVIEW COMPLETED: 11.19.97
REVIEWER: Douglas C. Throckmorton M.D.

0.0 RESUME

Nitric oxide is a locally released and active vasodilator. The current NDA proposes the use of inhaled nitric oxide (abbreviated I-NO in this review) in hypoxic respiratory failure in near- or full-term infants.

In support of this claim, the sponsor has submitted the results of four trials: the Neonatal Inhaled Nitric Oxide Study (NINOS); the Inhaled Nitric Oxide Study Group (INOSG) study; and the two studies sponsored by Ohmeda (INO-01/-02 and INO-03). The populations studied and the types and quality of the data collected during each of the trials varied considerably among these four trials. A list of the abbreviations used in this review is found in appendix one.

1. NINOS Trial

The NINOS trial demonstrated that I-NO administration is associated with a significant reduction in the incidence of death and/or the initiation of extracorporeal membrane oxygenation (ECMO), when compared with control subjects. This result, demonstrating efficacy of I-NO on the pre-specified, primary endpoint of the NINOS study, was driven almost entirely by a decreased rate of ECMO use in the I-NO group, as no effect of I-NO on mortality was detected. The effect of I-NO to reduce the use of ECMO, in turn, was primarily a reflection of the acute improvement in oxygenation caused by I-NO. Thus, the reduction in the incidence of the primary endpoint was largely a surrogate for improved oxygenation. No other clinically beneficial effect of I-NO was demonstrated.

With regards to safety, the NINOS trial was not designed to collect all adverse events, and important safety information was not collected, or is not available. No effect of I-NO on mortality was detected. There is a possible association between I-NO administration and pulmonary toxicity, as evidenced by increase incidence of pneumothoraces during I-NO administration. The administration of I-NO was also associated with higher peak NO_2 and methemoglobin levels.

2. INOSG trial

The INOSG trial demonstrated that administration of I-NO 80 ppm is associated with significantly improved oxygenation status after 20 minutes, relative to control subjects. This was the pre-specified primary endpoint.

Several aspects of the dataset for the INOSG trial raise serious questions about the trial blinding beyond the initial 20 minute period of exposure to study gas. The long-term results from the INOSG trial, including the data on the use of ECMO, should be regarded as being obtained from an open-label investigation. Within this constraint, the trial found that I-NO administration is associated with a decreased use of ECMO. No other clinically beneficial effect of I-NO was demonstrated.

With regards to safety, the INOSG trial was inadequate to address any aspects of long-term safety beyond mortality rate. There was no detectable effect of I-NO on mortality rate. In the INOSG trial, adminstration of I-NO, 80 ppm, was associated with a significant risk of elevated methemoglobin levels.

0.0 RESUME (cont)

3. Ohmeda 01-02 Trial

No effect of I-NO on the primary endpoint for the INO-01/ -02 trial was detected. The INO-01/ -02 trial was stopped before enrollment of the proposed number of subjects occurred, limiting its ability to detect significant differences in outcome. There was, however, a trend towards a reduction in the rate of ECMO in infants receiving I-NO when compared to controls. No significant effect of I-NO administration on any clinical outcome included as a secondary measure was detected.

Administration of I-NO acutely improves oxygenation, an effect which was dose-dependent between 5 and 80 ppm of I-NO.

With regards to safety, there was a non-significant increase in the number of deaths in the I-NO group, relative to control. Administration of I-NO 80 ppm was associated with a significant risk of elevated methemoglobin and NO_2 levels. The development of several laboratory abnormalities were also linked to the administration of I-NO. Finally, administration of I-NO was possibly associated with both acute and chronic pulmonary toxicity, as evidenced by increased rates of asthma, pneumothoraces, and the long-term need for supplemental oxygen in the I-NO group.

4. Ohmeda 03 trial

This trial was also hampered by the small number of subjects enrolled (14 total subjects). While efficacy was not specifically part of the proposal for this trial, the subjects were transferred to ECMO at rates similar to those of the subjects in the larger trials.

With regards to safety, no adverse events were seen which did not also occur in the other, larger, trials. No deaths occurred in the INO-03 trial.

Conclusions

Two of the trials submitted as part of the NDA met their pre-specified primary endpoints. In the NINOS trial, the administration of I-NO was associated with a significant decrease in the rate of death before 120 days and/or the initiation of ECMO. The INOSG trial demonstrated that I-NO causes a significant improvement in oxygenation after 20 minutes (the pre-specified primary endpoint). This endpoint is of insufficient clinical benefit to be a basis of an efficacy claim. In the INOSG trial, a second post-hoc analysis examined the receipt of ECMO in the two groups, and found a significant reduction in the initiation of ECMO in the I-NO group. The long-term INOSG datasets are incomplete, and this result should be considered to result from unblinded investigation. The third trial, the INO-01/ -02, failed to demonstrate any significant effect of I-NO on its pre-specified primary endpoint. There was a non-significant trend towards reduction in the use of ECMO in the INO-01/ -02 trial. The INO-03 trial was too small to contribute to discussions of efficacy.

The NINOS, INOSG and INO-01/ -02 trials demonstrated an acute effect of I-NO to improve oxygenation. This effect was durable and dose-dependent in the INO-01/ -02 trial.

No effect of I-NO on any clinically relevant endpoint was demonstrated or suggested, with the exception of a reduction in the use of ECMO. This reduction in ECMO use reflected the acute effects of I-NO to improve oxygenation, and may be nothing more than a surrogate for improved oxygenation, rather than a marker of durable clinical benefit. No effect of I-NO on the overall mortality rate was detected.

Several safety issues were identified as being possibly, probably, or definitely associated with the administration of I-NO. The most significant of these is a possible association with acute and chronic pulmonary toxicity. Additionally, the administration of 80 ppm I-NO was definitely associated with a markedly-increased incidence of methemoglobinemia and elevated NO_2 levels. The safety database was inadequate to exclude several potentially significant adverse events. There was also inadequate follow-up data available for identified adverse events.

Overall, I-NO was demonstrated to be effective (gauged by meeting the pre-specified endpoint with agreed-to clinical benefit) in one trial. This result was not overwhelming, and does not make up for the lack of any other demonstrated clinical benefit of I-NO. This absence of demonstrated clinical benefit comes from the four trials submitted as part of the NDA database, as well as data from other clinical sources, including publications and specific individual investigator INDs. In the absence of evidence of convincing clinical benefit in two randomized, double-blind trials, and with the association of I-NO administration to short- and long-term adverse events, NDA 20-845 is not approvable.

TABLE OF CONTENTS

Table of Contents (continued)

1.0 Materials Utilized in Review:

1) NDA 20-845 submission 6.16.96.

2) A copy of the NINOS data in SAS format forwarded by the NIH to Ohmeda, dated 11.1.96.

3) A copy of the INOSG and INO-01/ -02 data in SAS format from Ohmeda.

4) Copies of the published papers from the Neonatal Inhaled Nitric Oxide Study (NINOS) Group(1) and the Inhaled Nitric Oxide Study Group (INOSG)(2).

5) Letter from Dr. Joel Verter detailing discrepancies between the published NINOS Group article(1), and the data submitted as part of the IND (#4 above), dated 5.20.97.

6) IND submitted 5.13.94 by National Institute of Child Health and Human Development (NICHD) (Linda L. Wright, M.D., Project Manager and Monitor, Richard A. Ehrenkranz, M.D., Principle Investigator) for the treatment of term and near-term infants with hypoxic respiratory failure.

7) IND submitted by Ohmeda for PPHN.

8) IND submitted Ohmeda for ARDS.

9) IND Individual Investigator IND submitted by

10) IND Individual Investigator IND submitted by

11) IND Individual Investigator IND submitted by

12) Multiple other individual investigator INDs (total of 217 approved INDs as of 5.16.97).

13) Published articles from individuals holding individual-investigator INDs using inhaled I-NO in pediatric subjects. These are listed in section 5.2.2.1.

14) Transcript of the Cardiovascular and Renal Drugs Advisory Committee Meeting, 8.28.95, on Nitric Oxide.

15) Case Report Forms from the INO-01/ -02 trial for subject death, drop-out and serious adverse events.

16) Study summary data-sheets from the NINOS trial for all subjects.

17) General correspondence dated 8.22.97, detailing responses to questions concerning the INOSG trial. Also included were case summaries for the 5 deaths that occurred during the trial.

18) Long-term safety data from INO-01/ -02 trial, submitted 7.28.97.

1.1 Materials from NDA/IND

The NDA submission consists of 67 volumes containing the pre-clinical and clinical data on the effects of I-NO. Included as part of the clinical materials are summaries and data listings for four controlled clinical trials using I-NO in neonates with hypoxemic respiratory failure: 1) the Neonatal Inhaled Nitric Oxide Study (NINOS; Richard A. Ehrenkranz, M.D., Principle Investigator, Linda L. Wright, M.D., Project Manager and Monitor); 2) the Inhaled Nitric Oxide Study Group study (INOSG; Jesse D. Roberts, Jr., M.D., Principle Investigator); the combined INO-01/ -02 trial (Ohmeda; Dennis Davidson, M.D., Principle Investigator); and 4) the INO-03 trial (Ohmeda; Dennis Davidson, M.D., Principle Investigator).

Case report forms (CRFs) were submitted from the INO-01/ -02 trial for deaths, drop-outs and serious adverse events. The other two trials were planned and conducted by the principle investigator(s), and collected data on specific adverse events felt to be potentially associated with exposure to I-NO. Thus, no CRFs are available from the NINOS and INOSG trials. The NINOS trial report forms, which were completed on each infant for central data-entry, were also available.

Data sets for these trials submitted were also submitted in SAS format. Complete listings of the collected subject data from the NINOS and INO-01/ -02 trials were submitted. A partial listing of the INOSG data was submitted: 1) data on the acute effects of NO for both control and I-NO groups; 2) data on the effects of I-NO after 30 minutes in the subset of 'responding' I-NO subjects.

1.2 Related Reviews, Consults for the NDA

Consultation was obtained from the Division of Pulmonary Drug Products (HFD-570), regarding the clinical outcomes of the three major trials (NINOS, INOSG, and INO-01/ -02). This consultation, provided by Miriam Pina, M.D., is included separately.

1.3 Other Resources

1) An advisory committee met on 8.28.95 to discuss the use of I-NO in neonates. The minutes from that meeting were reviewed.

2) Individual INDs identified as containing efficacy data from large trials utilizing I-NO. Information within the individual INDs of relevance was reviewed.

3) An independent literature review was also conducted by this reviewer. Publications were identified using the names from the 217 individual investigator INDs approved by the FDA that had been approved as of 5.97. Publications were identified using Medline. The results of these published studies on the use of I-NO will be summarized in section 5.2.3 below, in the following order:

a. Twenty-three studies reporting the clinical effects of I-NO in neonates with hypoxic respiratory failure (most closely approximating the populations studied in the NINOS and INOSG trials);

b. Studies on the clinical effects of I-NO in neonates and infants with congenital heart disease and/or congenital diaphragmatic hernia; and

c. Miscellaneous other articles dealing with the mechanics of NO delivery or potential toxicities of I-NO as well as reviews of clinical I-NO use.

2.0 Background

Persistent pulmonary hypertension of the newborn (PPHN) is an important cause of cardiopulmonary failure and death in term and near-term infants. Its incidence has been estimated at between 4500 and 8000 cases per year in the US. PPHN has been defined as a syndrome developing in the first hours of life characterized by profound neonatal hypoxemia resulting from persistent or recurrent elevation of the pulmonary vascular resistance to a level exceeding the simultaneously measured systemic vascular resistance, producing hypoxic pulmonary vasoconstriction, pulmonary hypertension, and right-to-left cardiac shunting through the patent foramen ovale and/or patent ductus arteriosus. Clinically, these changes result in hypoxemia, hypercapnia and acidosis. PPHN can occur either as a primary condition, as in alveolar capillary dysplasia, or secondary to a variety of other clinical conditions, including: meconium aspiration; sepsis; pulmonary infections; or congenital diaphragmatic hernia. The diagnosis is made primarily using cardiac echocardiography, although angiography is performed in selective cases(3). Angiography is performed to distinguish PPHN from the uncommon entities which can be confused with PPHN: anomalous pulmonary venous return to the heart; severe cardiac outflow obstruction; and pulmonary hypoperfusion.

In the late 70's and early 80's, PPHN carried a very high mortality. More recently, with the development of extracorporeal membrane oxygenation (ECMO), High-Frequency Jet Ventilation (HFJV), and High-Frequency Oscillatory Ventilation (HFOV), survival rates have improved dramatically, even in patients with severe PPHN(4-6). Additional therapeutic interventions currently in use for PPHN include: intubation, hyperventilation with high-frequency jet ventilators; pharmacological alkalinization; sedation and paralyzation; and IV vasodilation. The latter therapy is associated with systemic hypotension. PPHN is now the most common diagnosis in infants who are referred for ECMO.

Inhaled NO (I-NO) has been proposed as a therapy for PPHN. *In vitro*, I-NO causes vasodilation by relaxing smooth muscle through the activation of the family of cGMP-dependent protein kinases. In the perinatal and neonatal circulation, I-NO is also thought to regulate basal pulmonary tone(7, 8). It has been suggested that I-NO production is stimulated by the act of respiration and/or the increase in PaO_2 that occurs during birth(9-11). Preliminary work in animals suggests that inhaled I-NO acts as a selective dilator of the pulmonary vasculature and is beneficial in reversing pulmonary hypertension. I-NO has been shown to reverse hypoxic pulmonary vasoconstriction, increase systemic oxygenation, and improve survival in lambs with persistent pulmonary hypertension. Preliminary clinical studies have also suggested that inhaled I-NO increases systemic oxygenation in infants with pulmonary hypertension(6, 12). Another hypothetical advantage of I-NO is that since it is administered by inhalation, it will most effectively dilate pulmonary blood vessels that are associated with the best ventilated lung, enhancing ventilation-perfusion matching.

2.1 Indication

The sponsor proposes inhaled I-NO ...'for use in conjunction with mechanical ventilation for the treatment of hypoxic respiratory failure in term and near-term neonates.' (NDA vol. 2.13, page 001204).

2.2 Important Information from Related INDs and NDAs and from Pharmacologically Related Agents

Many chemically distinct drugs share as a property the ability to cause vasodilation. Their mechanisms of action, where know, are equally diverse. A shared effect of several of the vasodilators, however, is that their effects are mediated, wholly or in part, through the release of NO. Examples of such compounds include the nitro-vasodilators (sodium nitroprusside, isosorbide-5-mononitrate) which act via release of NO_2 and prostacyclin, which indirectly stimulates NO release locally.

A major consequence of continued use of nitro-vasodilators is the development of tolerance, where-by the vessel no longer dilates in response to the agent(13, 14). During the tolerant phase, the effect of nitrates to lower blood pressure, as well as their anti-anginal properties, are greatly reduced, regardless of the dose used(15). Despite therapeutic plasma concentrations, loss of anti-anginal efficacy can be seen within the first 24 hours of continuous exposure to oral isosorbide-5-mononitrate. Following the characterization of tolerance, dosing of oral and transdermal nitrates were changed to include a 'nitrate-poor' period, to prevent the development of tolerance. There is no published data on the development of tolerance to I-NO.

2.3 Administrative History

Inhaled nitric oxide (I-NO) has been investigated by many individuals and by the current sponsor, Ohmeda. It has also been the subject of a meeting of the Cardiovascular and Renal Drugs Advisory Committee on 8.28.95. At that meeting, the adequacy of improved oxygenation as an endpoint to support an efficacy claim for I-NO was specifically discussed, and rejected with 4 yes votes and 14 no votes (page 275 of the meeting transcript).

Four trials were submitted in support of the current NDA. The National Institute of Child Health and Human Development (NICHD) conducted the neonatal inhaled Nitric Oxide Study (NINOS) under IND 'to evaluate the efficacy of I-NO in the treatment of term and near-term infants with hypoxic respiratory failure.' The trial was stopped on 5.2.96, after the Data Safety Monitoring Committee for the study concluded that the study had met it's primary outcome. The results of the investigations were then published (1). Dr. Wright, from the NICHD, has agreed that the results of the NINOS trials may be referred to by the FDA in support of the Ohmeda NDA, and has transmitted the data from the NINOS trial to Ohmeda (dated 11.1.96).

Simultaneous to the publication of the NINOS trial, the Inhaled Nitric Oxide Study Group (INOSG) reported the results of their trial, on the use of I-NO in the treatment of persistent pulmonary hypertension of the newborn(2). The data from this study has also been also been submitted in support of the present NDA.

A third set of clinical investigations was carried out by Ohmeda, for use in the treatment of persistent pulmonary hypertension in the neonate (PPHN) and for Acute Respiratory Distress Syndrome (ARDS), under IND and INE respectively. Shortly after the NINOS trial was closed, Ohmeda stopped their trials on 6.25.96, due to difficulty with patient recruitment. The INO-01 and INO-02 trials were ultimately combined into a single trial for submission to the FDA.

The INO-03 trial, a small safety trial with limited subject enrollment (14 total), was submitted separately.

Finally, many individual investigator INDs have been approved for the use of inhaled I-NO in both pediatric and adult populations (total of 217 approved INDs as of 5.16.97).

Chronology of key events and submissions

10.4.91 IND submitted by W. Zapol, MD, for compassionate use of I-NO in a single infant received by FDA. A separate protocol is also submitted for use of I-NO in adult respiratory distress syndrome (ARDS). Both protocols are reviewed and approved by R. E. Keenan, MD.

10.20.92 Meeting between Cardio-Renal Division of the FDA, , and W. Zapol, MD, concerning 'development of NO for treatment of acute pulmonary hypertension and acute pulmonary hypoxemia in children and adults.' Dr. Lipicky suggested the initiation of a controlled clinical trial to demonstrate efficacy and safety of I-NO, in addition to the study underway by Dr. Zapol.

6.22.93 Nitric oxide is designated an orphan drug (application

8.13.93 INOSG trial protocol received as part of discussion package from including ...'a copy of the protocol for the ongoing multicenter clinical trial sponsored by Massachusetts General Hospital'. This is the protocol for the INOSG trial. No formal FDA review is recorded.

12.13.93 INO-01/ -02 trial protocol submitted. Protocol, with revisions, is approved by N. Stockbridge, MD.

5.13.94 NINOS trial protocol submitted to the FDA. Protocol, with revisions, is approved by N. Stockbridge, MD.

12.26.94 Meeting between the Cardio-Renal Division of the FDA and Ohmeda to discuss clinical endpoints. FDA suggested the use of 'hard' endpoints such as mortality rather than physiological parameters.

6.5.95 Meeting between the Cardio-Renal Division of the FDA and multiple individual investigators using I-NO. Topics discussed included: possible competition between investigators for subjects; willingness of those present to join in one or two major trials; the potential utility of the oxygenation index (OI) as the basis for approval of I-NO in PPHN; and the trial size and number necessary for agency approval.

2.3 Administrative History (cont)

Chronology of key events and submissions (cont)

8.28.95 Cardiovascular and Renal Drugs Advisory Committee meeting to discuss investigation of I-NO in pediatric respiratory failure. The committee ultimately voted against the use of oxygenation parameters (in particular the OI) as a valid efficacy endpoint for I-NO trials in neonatal respiratory failure. The result was 14 votes against the use of improvement in oxygenation, especially changes in OI, as a primary efficacy endpoint, with 4 supportive votes, and 5 abstentions.

5.2.96 NINOS trial was halted at the recommendation of the trial's Data Safety and Monitoring Board, who determined that the study met it's primary combined endpoint after recruitment of 207 of the 250 projected subjects. Dr. Wright agreed that this data may be submitted in support of the Ohmeda NDA and forwarded the data-sets to Ohmeda.

6.25.96 INO-01/ -02 trial was stopped due to poor accrual after the NINOS trial is stopped.

2.27.97 The results of the NINOS and the INOSG studies were published(1, 2).

3.31.97 NINOS trial results and data were submitted to the FDA.

6.17.97 NDA 20-845 (Nitric Oxide) was submitted to the FDA.

7.28.97 Long-term safety data from INO-01/ -02 trial was submitted to the FDA.

9.16.97 NDA 20-845, for I-NO, was withdrawn by the sponsor ...'for business reasons and not due to concerns over product safety'.

2.4 Proposed Labeling

The sponsor proposes I-NO ...'for use in conjunction with mechanical ventilation for the treatment of hypoxic respiratory failure in term and near-term (≥34 weeks) neonates.'

The only contraindication proposed is for neonates 'known to be dependent on right-to-left shunting of blood.'

The recommended dose is 20 ppm: 'while doses of up to 80 ppm can be used, a 20 ppm dose is as likely to have an improvement in oxygenation with less risk of methemoglobinemia or higher NO_2 levels. Therefore, it is recommended that a constant dose of 20 ppm (I-NO) should be maintained... ' (NDA vol. 2.13 page 001904).

No drug interactions are identified in the draft label.

2.5 Foreign Marketing

No foreign marketing of I-NO has occurred.

3.0 Chemistry, Manufacturing, and Controls

Nitric oxide (NO) is generated from L-arginine by the action of NO synthase, which is activated by a variety of stimuli. In blood vessels, NO is released by endothelial cells and acts on nearby smooth muscle cells to cause vasodilation by activating the family of cGMP-dependent protein kinases.

No clinical implications of the manufacturing and control problems related to this NDA for inhaled NO (I-NO) were identified.

As proposed, I-NO will be provide in two strengths diluted in N_2: 100 and 800 ppm. The infants are all ventilator-dependent, so that the I-NO/N_2 gas mixture will be diluted with the O_2/room air mixture normally given to the infant just prior to ventilation to the infant. The 100 ppm tank is anticipated to be used only during weaning, when very low concentrations of I-NO are needed. Due to the use of N_2 as a carrier, and the need to use high rates of I-NO gas flow to minimize the time NO is in contact with O_2 in the lines, the administration of I-NO results in a decrease in the maximum FiO_2 which can be delivered to the subject. These maximums are shown below using the 5, 20, and 80 ppm I-NO.

Maximum diluted FiO_2 delivered during use of 100 ppm and 800 ppm I-NO cylinders. [a].

Delivered NO Dose (ppm)	Maximum delivered FiO_2 800 ppm tanks of I-NO	Maximum delivered FiO_2 100 ppm tanks of I-NO
5	0.99375	0.95
20	0.975	0.80
100	0.90	0.20

a. Data from Ohmeda correspondence dated 9.16.97, page 3.

4.0 Pharmacology/pharmacokinetics

Please see the pharmacologist's review for further details of the pharmacology and pharmcokinetics of I-NO. *In vivo*, I-NO has been shown to be a selective pulmonary vasodilator, resulting in a dose-dependent reduction in pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR). The rapid inactivation of I-NO by hemoglobin is thought to prevent I-NO from having significant systemic effects.

4.0.1 Non-clinical pharmacology/pharmacokinetics

In pre-clinical studies, I-NO has been shown to cause vasodilation of the pulmonary vasculature in several species following a variety of vasoconstrictive stimuli: hypoxia; monocrotaline-induced pulmonary injury; and infusions of U-46619 (thromboxane analogue), lipopolysaccharide, angiotensin II, bradykinin, or microbeads into the pulmonary circulation. This effect of I-NO is dose-dependent and occurs at I-NO concentrations of 5-80 ppm. Inhaled I-NO up to 160 ppm has been shown to be free of systemic hemodynamic effects in animal models.

For example, in an ovine model of PPHN, inhaled I-NO (6-100 ppm) causes a dose-dependent decrease in PAP and PVR without an effect on systemic hemodynamics(16, 17). In a second study, using a model of pulmonary hypertension, the authors were able to demonstrate improved survival for lambs treated with I-NO, 80 ppm, for 23 hours.

Local concentrations of I-NO, since it is produced locally, are impossible to directly measure. As such, no comparison between the proposed concentrations of inhaled nitric oxide and the local concentrations, are possible. An estimated 90% of the I-NO is absorbed and combines with oxyhemoglobin to form methemoglobin and nitrate. Nitrate is predominantly cleared from the plasma by the kidney at rates approaching glomerular filtration. The majority of methemoglobin is reduced to hemoglobin under normal circumstances by the action of NADH-dependent methemoglobin reductase in red blood cells. No significant pharmacological effect of I-NO, aside from production of methemoglobin, has been described.

4.0.2 Clinical pharmacology/pharmacokinetics

The clinical pharmacology of I-NO will be included in the discussion of the clinical literature on the use of I-NO in section 5.2.2.1 and 5.2.2.2 below.

4.1 Toxicology, genotoxicity, & carcinogenicity

In two studies performed by the sponsor to investigate the toxic effects of I-NO, anesthetized dogs were exposed to I-NO for 6 hours at a concentration of 1, 80, 160, 320 and 640 ppm. The major toxicity detected was methemoglobinemia, first detected at 160 ppm. The % methemoglobinemia was progressive at higher concentrations, up to a maximum average of 78% of all hemoglobin at 640 ppm of I-NO. Electrocardiographic changes were also noted, including ventricular premature depolarizations, sinus tachycardia (at 80 and 320 ppm), junctional rhythm (80 and 160 ppm), and R on T phenomenon (640 ppm). These changes were attributed to anesthesia and intubation, as a repeat study using conscious animals with permanent tracheotomies demonstrated no differences between placebo and I-NO groups.

The sponsor has also examined the effect of chronic exposure of rats to 200 ppm I-NO. Per their summary, an increase in interstitial edema was the only consistent finding in the rats exposed to I-NO (NDA vol. 2.5, page 84).

Exposure of beagles to I-NO, 1.76 ppm, for 61 months had no serious pulmonary effects, although an increased number of alveolar fenestrations were observed in the treated dogs(18).

Concerns regarding specific toxicities have also been examined in animal. One potential safety concern is an adverse effect of I-NO on the production of pulmonary surfactant. An effect of I-NO to alter surfactant production has been reported in rats(19).

Another possible toxicity is an effect of I-NO on cerebral hemodynamics. This effect was examined in 8 sheep, where acute pulmonary vasodilation caused no change in cerebral blood flow or cerebral oxygen consumption(20).

The genotoxicity of I-NO has been investigated in a variety of systems, with conflicting results. Nitric oxide has demonstrated both negative and positive genotoxic potential in a battery of *in vitro* and *in vivo* test systems. In vitro assay have administered NO gas directly to the culture medium or have used an NO donor such as spermine-NO complex or nitroglycerin to deliver the NO to the test system. Positive mutagenesis has been demonstrated for NO in the bacterial reverse mutation assay using the *S. typhimurium* TA1535 test strain. Conflicting findings have been demonstrated in various *E. coli* strains. In mammalian cells, NO has both caused mutagenesis in cells lines (AD293 cells and TK6 human lymphoblasts cells) and failed to cause mutagenesis in other cells lines (CHO cells, human bronchial epithelial cells). *In vivo* inhalation of NO by Sprague-Dawley rats for 8 hours (27 ppm) followed by primary culture of the lung cells demonstrated increased mutation to oubain resistance and no increase in the frequency of chromosomal aberrations. In summary, the sponsor states that ... 'NO has demonstrated positive genotoxic potential in bacterial and mammalian cell culture and after *in vivo* inhalation exposure and analysis of lung cells in primary culture.' (NDA volume 2.5, page 002505).

No information regarding the potential carcinogenicity of I-NO is available.

5.0 Description of Clinical Data Sources

1) NDA 20-845 submission 6.16.96.

2) A copy of the NINOS data in SAS format forwarded by the NIH to Ohmeda, dated 11.1.96.

3) A copy of the INOSG and INO-01/ -02 data in SAS format from Ohmeda.

4) Copies of the published papers from the neonatal inhaled Nitric Oxide Study (NINOS) Group(1) and the Inhaled Nitric Oxide Study Group (INOSG)(2).

5) Letter from Dr. Joel Verter detailing discrepancies between the published NINOS Group article(1), and the data submitted as part of the IND (#4 above), dated 5.20.97.

5.0 Description of Clinical Data Sources (cont)

6) IND submitted 5.13.94 by National Institute of Child Health and Human Development (NICHD) (Linda L. Wright, M.D., Project Manager and Monitor, Richard A. Ehrenkranz, M.D., Principle Investigator) for the treatment of term and near-term infants with hypoxic respiratory failure with I-NO.

7) IND Ohmeda for the treatment of PPHN with I-NO.

8) IND Ohmeda for the treatment of adult respiratory distress syndrome (ARDS).

9) IND Individual Investigator IND submitted by

10) Multiple other individual investigator INDs (total of 217 approved INDs as of 5.16.97)

11) Published articles from individuals holding individual-investigator INDs using inhaled I-NO in pediatric subjects. These are listed in section 5.2.2.1.

12) Transcript of the Cardiovascular and Renal Drugs Advisory Committee Meeting, 8.28.95, on Nitric Oxide.

13) Case Report Forms from the INO-01/ -02 trial for subject death, drop-out and serious adverse events.

14) Case Report Forms from the INO-01/ -02 trial for specific adverse events, requested by this reviewer.

15) Long-term safety data from the INO-01/ -02 trials, submitted 7.28.97.

16) Letter withdrawing the NDA 20845, dated 9.16.97.

5.1 Primary Source Data (Development Program)

In support of this claim, the sponsor has submitted the results of four trials. The NINOS trial examined the effects of I-NO in neonates with hypoxic respiratory failure (with and without pulmonary hypertension). Subjects were randomized to either control gas or I-NO, 20 ppm. The study's primary endpoint was the occurrence of either death and/or the initiation of ECMO prior to discharge or 120 days. Subjects who failed to respond to the low-flow study gas (I-NO 20 ppm or control), were eligible to receive high-flow study gas (I-NO 80 ppm or control).

The INOSG trial examined the acute effects of I-NO, 80 ppm, on oxygenation and hemodynamics in hypoxic neonates with pulmonary hypertension. The primary endpoint of the trial was the effect of I-NO on oxygenation after 20 minutes, compared with control gas.

The INO-01/ -02 trial examined the effects of a range of I-NO concentrations in neonates with hypoxic respiratory failure and pulmonary hypertension, and compared them in randomized, double-blinded, fashion with control gas. The primary endpoint of the study was the occurrence of any one of four adverse clinical events: initiation of ECMO; death; the occurrence of risk factors for abnormal neurological sequelae; and bronchopulmonary dysplasia.

The INO-03 trial was designed to extend the safety data in the use of I-NO in infants with pulmonary hypertension. Efficacy data on the primary endpoint used in the INO-01/ -02 trial was also collected.

Each of the trials collected different data on the enrolled subjects. The table below summarizes the materials available from each of the trials for this review. As can be seen from this summary table, two trials (INO-01/ -02 and -03) collected routine adverse events and clinical laboratory values. The INO-03 trial has a very small number of subjects (14). The effect of this is to limit the information that can be obtained from the database as to the effect of I-NO on unanticipated adverse events (e.g., hypokalemia, anemia, rash).

Table 5.1.1 Safety data collected in the NINOS, INOSG, INO-01/ -02 and INO-03 trials[a].

Study	Deaths	All Adverse Events	Selected Adverse Events	NO_2 Levels	MetHgb Levels	All Clinical Lab Values	Case Report Forms	Case Summaries	Long-term Follow-up
NINOS	Y	N	Y	Y	Y	N	N	Y	Pending
INOSG	Y	N	Y	N	Partial	N	N	Partial	N
INO-01/ -02	Y	Y	Y	Y	Y	Y	Y	Y	Y
INO-03	Y	Y	Y	Y	Y	Y	Y	Y	N

a. From NDA, volume 2.50, page 339110.

5.1.1 Study type and design/patient enumeration

Table 5.1.1.1 Enumeration of subjects enrolled from NINOS, INOSG, INO-01/ -02 and INO-03 trials. For details, see individual study reviews in section 6.0.

Trial	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
NINOS	121 (117 tx'd)		114	55[a]	114 (113 tx'd)
INOSG	28			30	30
INO-01/02	41	41	36	37	114
INO-03	0		14		14
Total	186 (tx'd)	41	164	122	271 (tx'd)

a. A subset of the subjects in NINOS who failed to respond to I-NO 20 ppm were administered I-NO 80 ppm (55/114).

5.1.2 Demographics

The demographics of the subjects in the trials submitted as part of the NDA are summarized in table 5.1.2.1 below. Following that are tables detailing the demographics of the individual studies.

Table 5.1.2.1 Combined demographics from the NINOS, INOSG, INO-01/ -02, and INO-03 trials[a].

Demographic Parameter	Control	I-NO
Total	186	271
Sex		
Male	121 (63%)	146 (54%)
Female	67 (37%)	123 (46%)
Race		
White	102 (55%)	148 (55%)
Black	34 (18%)	56 (21%)
Hispanic	31 (16%)	41 (15%)
Asian	1 (<1%)	5 (3%)
Other	13 (7%)	18 (7%)
Missing	5 (3%)	4 (1%)
Age at start of tx gas (hrs)	36.2	33.1
Birth weight (kg)	3.4	3.45
Gestational age (weeks)	39.2.	39.7

a. Data from electronic datasets and NDA volume 2.29.

Table 5.1.2.2 Demographics of subjects in the NINOS trial[b].

Variable	Control	I-NO	p-value[a]
Total	121	114	
Sex			
Male	76 (63%)	63 (55%)	0.24
Race[b]			
White	72 (60%)	70 (61%)	
Black	19 (16%)	19 (17%)	
Hispanic	17 (14%)	13 (11%)	
Other	11 (9%)	9 (8%)	
Missing	2 (<1%)	3 (3%)	
Age at start of tx gas (days)	1.7±2.3	1.7±1.8	0.78
Birth Weight (kg)	3.4±0.6	3.5±0.6	0.19
Gestational Age (weeks)	38.9±2.2	39.3±1.8	0.16

a. p value calculated using unadjusted chi-square.

b. Data from NDA volume 2.14, section 11.2.2. Data shown is for all subjects randomized, including 4 control infants and 1 I-NO who did not receive study gas after being randomized.

5.1.2 Demographics (cont)

Table 5.1.2.3 Demographics of the 58 subjects in the INOSG trial[b].

Demographic Parameter	Control	I-NO	p-value[a]
Total	28	30	
Sex			
Male	18 (64%)	16 (53%)	0.43
Female	10 (36%)	14 (47%)	
Race			
White	13 (46%)	11 (37%)	0.72
Black	4 (14%)	8 (27%)	
Hispanic	4 (14%)	7 (23%)	
Asian	2 (7%)	1 (3%)	
Other	2 (7%)	2 (7%)	
Missing	3 (11%)	1 (3%)	
Age at start of tx gas (hrs)	31.6±29.5 (2.7 to 147 hrs)	38.6±42.6 (0.6 to 189.4 hrs)	0.5
Birth weight (kg)	3.56±0.62 (2.5 to 4.68 kg)	3.44±0.58 (2.4 to 4.77 kg)	0.49
Gestational age (weeks)	40.1±1.2 (37 to 42)	39.8±1.5 (37 to 42)	0.39

a. p value calculated using unadjusted chi-square.
b. Data from NDA volume 2.16, section 11.2.2.

Table 5.1.2.4 Demographics of the subjects in the INO-01/ -02 trial[a].

Demographic Characteristic	Control	I-NO			I-NO
		5 ppm	20 ppm	80 ppm	Pooled
Total	41	41	36	37	114
Sex					
Male	27 (66%)	19 (46%)	20 (56%)	19 (51%)	58 (51%)
Female	14 (34%)	21 (54%)	16 (44%)	18 (49%)	56 (49%)
Race					
White (%)	20 (49%)	24 (59%)	18 (50%)	17 (46%)	59 (52%)
Black (%)	11 (27%)	7 (17%)	9 (25%)	9 (24%)	25 (22%)
Hispanic (%)	10 (24%)	8 (20%)	8 (22%)	4 (11%)	20 (18%)
Asian (%)	0 (0%)	1 (2%)	0 (0%)	3 (8%)	4 (4%)
Other (%)	0 (0%)	1 (2%)	1 (3%)	4 (11%)	6 (5%)
Age in hours[b]	26±18	22±14	24±16	27±20	24±17
Birth Weight (kg)	3.40±0.5	3.5±0.5	3.4±0.6	3.4±0.5	3.40±0.5
Gestational age in weeks	39.7±1.8	40.2±1.5	39.3±1.6	39.9±1.5	39.8±1.6
Mother's age in years	27.1±6.3	25.6±6.0	26.0±6.9	25.8±6.6	25.8±6.4

a. Data from NDA volume 2.17, page 083608.
b. Data shown is mean±standard deviation.

5.1.2 Demographics (cont)

Table 5.1.2.5 Demographics of the subjects in the INO-03 trial[a].

Demographic Characteristic	Control	I-NO			I-NO
		5 ppm	20 ppm	80 ppm	Pooled
Total	0	4	8	2	14
Sex					
Male		4 (100%)	4 (50%0	1 (50%)	9
Female		0 (0%)	4 (50%)	1 (50%)	5
Race					
White (%)		3 (75%)	4 (50%)	1 (50%)	8
Black (%)		0 (0%)	3 (38%)	1 (50%)	4
Hispanic (%)		0 (0%)	1 (13%)	0 (0%)	1
Other (%)		1 (25%)	0 (0%)	0 (0%)	1
Age in hours		48±22	24±12	21±21	
Gestational age in weeks		39.8±2	39.6±2	38.5±2	
Mean Weight (kg)		3.9±0.8	3.3±0.5	3.2±0.4	
Mother's age in years		27.8±8	27.4±6	24±0	

a. Data from NDA volume 2.31, page 389808.

5.1.3 Extent of exposure (dose/duration)

A total of 278 subjects received I-NO during the four trials submitted in this NDA, including the 271 randomized to receive I-NO, listed in the demographics tables above. In the NINOS trial, four subjects in the control group and one subject in the I-NO group did not received study gas, and so are not included in this table (or the safety analysis). Additionally, 7 subjects randomized to control were administered I-NO, and 1 subject randomized to I-NO received control gas. These subjects will be discussed separately below. The INO-03 trial enrolled only 14 subjects before being stopped, and so represents a small fraction of the total safety database.

Table 5.1.3.1 Enumeration of subjects from NINOS, INOSG, INO-01/ -02 and INO-03 trials exposed to I-NO.

Trial	Control	I-NO 5 ppm	I-NO 10 ppm	I-NO 20 ppm	I-NO 40 ppm	I-NO 80 ppm	I-NO 100 ppm	Combined I-NO
NINOS[a]	110	1	1	43	1	82	2	120 (130)
INOSG[b]	28					30		30
INO-01/02	41	41		36		37		114
INO-03	0			14				14
Total	186	42	1	93	1	149	2	278 (288)

a. All subjects in the I-NO group in NINOS were first exposed to 20 ppm. A subset of the subjects who did not respond were then given I-NO, 80 ppm. Small numbers of subjects also received either more, or less, then the intended 20 or 80 ppm (protocol violations).

b. Three subjects in the INO-01/ -02 trial also received the incorrect dose of treatment gas.

The extent of exposure to study gas is summarized below.

Table 8.0.2. Median duration of exposure to treatment gas (either N_2, O_2, or NO) from NINOS, INOSG, INO-01/ -02 and INO-03 trials.[a]

Study	Control Group	I-NO Group
NINOS	2 hours	40 hours
INOSG	20 minutes	48 hours
INO-01/ -02	34 hours	38 hours
INO-03	No control group	91.2 hours

a. From NDA volume 2.50, page 339210.

5.2 Secondary Source Data

Next, the other available data on the use of I-NO in neonates will be reviewed: other large clinical trials using I-NO in neonates; and the published literature on I-NO use. The published literature was searched for pertinent articles where the use of I-NO was reported. These materials, which are summarized below, are especially important for identifying potential safety concerns to be examined in the NDA database. They are also critical for addressing issues for which no data exists in the NDA (genotoxicity, carcinogenicity).

These published materials were considered secondary source data for the following reasons: absence of available primary data on individual subjects; absence of available case report forms; inadequate experimental detail regarding treatment; and lack of control groups (in almost all published studies). Their use in establishing the efficacy of I-NO is limited by the frequent lack of controls and blinding, and by the bias against the publication of negative efficacy data.

5.2.1 Other studies

There have also been six large, randomized trials of I-NO for neonates that are known to this reviewer. These are summarized below. The first three studies were submitted as part of the NDA. The results from the other three studies are not yet published, although preliminary results are available through the INDs under which one of the three studies were performed and are discussed below (no information regarding the status of the French study is available).

Table 5.2.2.1.1 Characteristics of randomized trials of I-NO for neonates.

Study	Population	Design	Endpoint	Subject #	Status
NINOS	Hypoxemia	I-NO vs. placebo gas (O_2)	ECMO and/or Death	235	Completed & Published (1)
INOSG	PPHN	I-NO 80 ppm vs. placebo gas (N_2)	Failure to improve oxygenation	60	Completed & Published (2)
INO-01/ -02 (Ohmeda)	PPHN	I-NO 5, 20, and 80 ppm vs. placebo gas (N_2)	Combined endpoint[a]	155[b]	Stopped early[b]
Children's Hospital, Denver	PPHN	I-NO/CMV vs. HFOV[c]	Improvement in oxygenation	288	Unpublished
Children's Hospital, Boston	PPHN	I-NO vs. placebo gas (O_2).	ECMO or Death	100	Pending publication (21)
French Study	Hypoxemia	I-NO 10 ppm vs. no study gas (control)	OI at the end of 2 hours	400	Unpublished

a. The INO-01/ -02 endpoint was the incidence of any one of the following: death; initiation of ECMO; abnormal neurologic sequelae; and bronchopulmonary dysplasia.

b. The INO-01/ -02 trial was stopped due to difficulties in enrollment following the publication of the NINOS results.

c. CMV = conventional mechanical ventilation. HFOV= high-frequency oscillatory ventilation.

1. Children's Hospital, Boston

This study, performed under IND examined the effect of I-NO on the incidence of ECMO or death, and is pending publication(21). The study population included hypoxic neonates with PPHN confirmed by echocardiography. Previous treatment with surfactant or high-frequency ventilation was allowed. Infants were randomized to either standard therapy vs. I-NO, which was started at 80 ppm. Infants were randomly assigned, but the investigators were not blinded as to the group assignments. A total of 23 control and 26 I-NO infants were enrolled, with an average baseline OI of 29 in controls and 30 in the I-NO group. The results of the study are summarized below. The only difference between the two groups which was suggested by the author was a decreased risk of seizures or intracranial hemorrhage in the I-NO group. This difference was not statistically significant. No difference in the rate of death or ECMO was detected. Dr. Wessel noted in his letter accompanying this data that a follow-up study of 90 subjects, including those reported below, did show decreased use of ECMO in the I-NO group. No data in support of this assertion were submitted.

Table 5.2.2.1.2 Preliminary results from the IND 38910 study[b].

Endpoint	Control (n=23)	I-NO (n=29)
Death	2 (9%)	2 (8%)
ECMO	8 (35%)	8 (31%)
Median days on ventilator	10	9
Median days on supplement O_2	12	13
% requiring home O_2	0	0
Seizures or ICH[a]	8	4

a. ICH = intracranial hemorrhage

b. Data from Annual Report for IND 38,910, submitted by Dr. David Wessel 6.11.97.

5.2.1 Other studies (cont)

2. Children's Hospital, Denver

This study, performed under IND compared the effect of I-NO with that of HFOV on the incidence of treatment failure, as determined by changes in oxygenation. Subjects with hypoxemia and echocardiographic evidence of PPHN were eligible. The average OI at baseline was 47 in the HFOV group and 49 in the I-NO group. Previous treatment with surfactant or high-frequency ventilation was allowed. Subjects were randomized in open-fashion to either I-NO, 20 ppm, versus HFOV. At the end of 2 hours, if oxygenation did not improve the infants were eligible for cross-over to combination therapy (I-NO + HFOV). A total of 205 infants were enrolled before the trial was stopped by the data and safety monitoring board because no statistical difference was detected between the initial treatment groups to that point with regards to changes in oxygenation. Projections at that point also suggested little likelihood of detecting differences with continued patient enrollment. No deaths occurred during the study. The rate of improvement in oxygenation was similar between the I-NO and HFOV groups, and there were no differences in the rates of air leak, duration of mechanical ventilation, or oxygen requirement after 28 days. The improvement in oxygenation after the combination of HFOV + I-NO was significantly more that either I-NO or HFOV alone. The authors concluded that the combination of HFOV + I-NO was more effective than either therapy alone at improving oxygenation.

5.2.2 Literature

Three approaches were used to identify relevant published literature relevant to the current submission.

First, an independent literature review was conducted by this reviewer. Publications were identified searching Medline using the names from the 217 individual investigator INDs approved by the FDA that had been approved as of 5.97.

Second, a keyword search of Medline was performed by this reviewer. Terms used in the search included: nitric oxide; pulmonary hypertension; human clinical trials.

Third, the bibliographies of recent review articles were screened for references to clinical trials (e.g., (4, 22, 23)).

Finally, the sponsor has provided a literature review, which was cross-referenced with the above reviews to assure completeness. The cut-off for consideration of articles in this NDA was approximately April of 1997.

5.2.2.1 Efficacy summary from published literature

The efficacy of I-NO in pulmonary hypertension has been examined by many individual investigators. The results of published studies on the use of I-NO are summarized below in the following order:

1. Twenty-six studies reporting the clinical effects of I-NO in neonates with hypoxic respiratory failure (most closely approximating the populations studied in the NINOS and INOSG trials).

2. Eight studies on the clinical effects of I-NO in neonates and infants with congenital heart disease and/or congenital diaphragmatic hernia.

3. Miscellaneous other articles dealing with the mechanics of I-NO delivery or potential toxicities of I-NO. Review articles summarizing the effects of I-NO will be listed here.

Articles on the use of I-NO in adults, and trials where the majority of the infants had congenital diaphragmatic hernia (CDH) were not reviewed.

5.2.2.1a Studies with I-NO in neonates with hypoxic respiratory failure without congenital heart disease

The table below summarizes the results of studies using I-NO in neonates with hypoxic respiratory failure, without structural heart disease other than patent ductus arteriosus and intra-cardiac shunts at the level of the atria.

Table 5.2.2.1a.1 Published studies on the use of I-NO in neonates with hypoxic respiratory failure.

Study	Population	N[a]	Dose of I-NO	Results
(1)	NINOS Trial, part of NDA submission			
(2)	INOSG Trial, part of NDA submission			
(6)	Neonates with PPHN	6	24, 40, 80 ppm	Postductal PaO_2 increased in 5/7 trials with 80 ppm I-NO (5/6 subjects) O_2 saturation increased in 5/6 subjects acutely (I-NO stopped after 30 minutes) No change in BP or $PaCO_2$ after I-NO No increase in methemoglobin levels after I-NO
(12)	Neonates : 4 with PPHN 5 MAS and/or sepsis	9	10-20 ppm	OI fell 66% (60±12 to 20±3) after 30 mins of I-NO Aa/-O_2 and PaO_2 also improved significantly 6/9 subjects recovered without ECMO or evidence of chronic lung disease
(5)	Neonates with PPHN who were candidates for ECMO: 3 MAS 3 sepsis 2 CDH 1 asphyxia	9 consecutive subjects	20 ppm for 4 hrs then 6 ppm for 20 hours	All oxygenation parameters improved in all subjects acutely and improvements were sustained for 24 hours of I-NO tx OI fell 58% (77±25 to 32±8) after 30 minutes of I-NO and 81% after 24 hours (77±25 to 14.6±3.7) Echocardiography showed improved cardiac function after 24 hours. In the first 24 hours, I-NO had no effect on systemic BP Heart rate fell 174±8 to 152±12 8/9 subjects had long-term clinical improvement without ECMO. 1 subject died of neurologic complications 1 subject responded initially, then declined and died after receiving ECMO.
(24)	Neonates referred for severe PPHN (PaO_2 <50 mmHg): 9 CDH 17 MAS 10 pneumonia 3 other	30	3-100 ppm	12/30 showed lasting improvement in oxygenation indices 13/30 transient improvement in oxygenation indices Authors state that 'NO apparently cut out a 15% segment from the patient group' who would otherwise have received ECMO which would not have responded to HJOV[d] One subject had a precipitous decline in BP following I-NO. No increased clinical bleeding seen in I-NO group

a. Number of subjects
b. arterial/alveolar oxygen ratio
c. MAS: Meconium Aspiration Syndrome, CDH: Congenital Diaphragmatic Hernia
d. HFOV: High Frequency Oscillatory Ventilation

5.2.2.1a Studies with I-NO in neonates with hypoxic respiratory failure without congenital heart disease (cont)

Table 5.2.2.1a.1 Published studies on the use of I-NO in neonates with hypoxic respiratory failure (cont)

Study	Population	N*	Dose of I-NO	Results
(25)	Neonate with hypoxic respiratory failure and severe right-to-left cardiac shunt following sepsis and intraventricular bleed.	1	20 ppm	OI improved from 76 to 5 following I-NO for 1 hour. Subject ultimately extubated and discharged home.
(26)	Neonates with PPHN 8 MAS 2 sepsis 5 idiopathic	13 consecutive neonates 7 had previously failed tolazoline or prostacyclin all received HFOV	20 ppm for 4 hrs then 6 ppm for 20 hrs	12/13 infants responded with improved oxygenation Average OI decreased 41% in 30 minutes (51 to 21) and 70% in 24 hours. All survivors (12/13) had responded by the end of 12 hours of I-NO
(27)	Near-term neonates with PPHN 5 MAS 3 CDH 5 PPHN 12 Sepsis, RDS, Misc. Few subjects received HFOV pre-I-NO	25 consecutive neonates	20 ppm	23/25 responded with improved oxygenation parameters after 30 minutes OI decreased following 20 mins I-NO, (86±16 to 21±2). PaO_2 increased following 20 mins of I-NO (33±3 to 93±8 mmHg). 12/23 responders failed to sustain clinical improvement 6/12 of these non-responders died 4/12 had PPHN Paper does not detail use of ECMO
(28)	10 term infants with PPHN 9 MAS 1 pneumonia 6/10 surfactant treated before I-NO	10 neonates	10-40 ppm	Median PaO_2 after 30 mins rose from 49 to 75 mmHg (p=0.005). OI fell from 37 to 21 (p=0.005). Systemic arterial pressure rose (mean 46.5 mmHg to 54.6 mmHg) Maximum NO_2 level 2.5 ppm 7/10 survived, 1 pulmonary hemorrhage, 2 progressive hypoxemia 1/10 received ECMO (later died)
(29)	All with RDS All surfactant treated	23 pre-term infants with RDS, treated for 15 minutes mean age 28+/- 0.6 wks	5 or 20 ppm	Both concentrations of I-NO caused an acute rise in PaO_2, SaO_2, and SpO_2 No differences between the two groups in clinical outcomes were detected. 18/23 infants left hospital 5 died due to "lethal combination of multiorgan abnormalities"
(30)	17 neonates with hypoxemia 16 with pulmonary hypertension 9 MAS 2 PPHN 1 Pulmonary hypoplasia	17 neonates Prospective, non-blinded randomized, cross-over design No surfactant or HFOV	20-80 ppm vs. control	4/9 in I-NO group initially responded with increased PaO_2 17/17 ultimately failed due to hypoxemia and I-NO was stopped 12/17 ultimately received ECMO, 6 control, 6 I-NO 1 control and 2 I-NO subjects died

5.2.2.1a Studies with I-NO in neonates with hypoxic respiratory failure without congenital heart disease (cont)

Table 5.2.2.1a.1 Published studies on the use of I-NO in neonates with hypoxic respiratory failure (cont)

Study	Population	N*	Dose of I-NO	Results
(31)	5 CDH 3 MAS 2 Pneumonia 4 surfactant treated	10 'ECMO candidates', mean OI 51.9	20 to 80 ppm	8/10 ultimately required ECMO 5/10 no improvement in oxygenation with NO 3/10 transient improvement only 1/10 died (after ECMO) Safety: no alteration in serial coagulation parameters no change in systemic blood pressure following I-NO
(32)	50 subjects (term and pre-term): 22 with 25<OI<40 11 control/11 I-NO 28 OI>40, all received I-NO 22 pneumonia 8 MAS 7 CDH	Newborns with OI: 1. 25-40, randomized to I-NO versus control 2. >40, received NO	20 ppm versus control	Acute response to I-NO predicted ultimate clinical outcome: 87% of those who acutely lowered OI to <40 maintained response 90% of those who did not acutely lower OI required ECMO or died 23/33 subjects with OI>40 responded acutely. Of these, 3 later relapsed. 10/33 subjects with OI >40 did not respond acutely. One of these later improved clinically Among patients with OI <40, I-NO acutely improved PaO_2 and OI versus control. Overall mortality rate 26% (no breakdown by I-NO therapy). Gestational age did not predict responsiveness and premature infants responded equally to term infants in terms of oxygenation to I-NO
(33)	2 MAS 5 CDH 2 pneumonia/sepsis	10 neonates on ECMO	20-80 ppm	6/10 oxygenation improved 9/10 survived No effect of I-NO on coagulation parameters seen No GI, pulmonary or CNS bleeding seen No dose-response detected
(34)	7 CDH 7 MAS 4 Sepsis 2 RDS	23 consecutive infants referred for ECMO, no structural heart disease OI>20 22/23 received surfactant	5-80 ppm	1 subject had reversible decrease in systemic BP to I-NO 20 ppm No dose-response effect at doses used (given in random order) 13/23 infants had initial response to I-NO 11 of these 13 had PPHN by echo 1/23 responded to the third trial of I-NO 9/23 failed to respond 3 of these had PPHN by echo 2/23 died (1 responder, 1 non-responder) Infants with R to L shunt less likely to respond to I-NO
(35)	3 MAS 1 Potter's Syndrome 1 Anomalous Venous Return 2 CDH	7 neonates (out of 14 total subjects)	15-80 ppm	6/7 improved PaO_2 within 60 minutes 2/7 died 1/7 infants had methemoglobin level of 5.5% on 80 ppm I-NO

5.2.2.1a Studies with I-NO in neonates with hypoxic respiratory failure without congenital heart disease (cont)

Table 5.2.2.1a.1 Published studies on the use of I-NO in neonates with hypoxic respiratory failure (cont)

Study	Population	N*	Dose of I-NO	Results
(36)	All infants referred for ECMO rescue for PPHN without congenital heart disease or CDH or chromosomal defect	16 subjects given I-NO compared with 21 historical controls who met criteria for need for ECMO	25-50 ppm vs. No I-NO	In group 1 (no I-NO) 16/21 required ECMO (72%) In group 2 (I-NO) 4/16 required ECMO (25%) % survival without interventricular hemorrhage or chronic O_2 was higher in Group 2 Mortality unchanged (4 deaths in group 1, 0 in group 2)
(37)	10 PPHN 1 MAS 4 RDS 1 sepsis 1 CDH 3 idiopathic PPHN	10 subjects being discontinued from I-NO when OI falls to <10	4.9 ± 0.8 ppm before being D/C'd	Shortly after NO withdrawal (time not specified): no change in BP, HR, CO decreased SPO_2 (97.7/0.5 to 93.9/0.5), PO_2 (71.3/4.8 to 52.6/4.2)
(38)	12 RDS 8 PPHN	17 hypoxic neonates some pre-term	Mean 20 ppm	OI decreased after starting NO (49/19 to 11/9) in subjects with extrapulmonary shunting (EPS) 40/11 to 20/13 in non-EPS subjects Mean pulmonary blood flow velocity (MPBFV) increased in EPS subjects (18/4 to 29/8) but not in non-EPS (19/12 to 21/11)
(39)	1 idiopathic PPHN	Single infant with idiopathic PPHN and Down's Syndrome	20 - 80 ppm	Transient response to I-NO 20-40 ppm, no response to 80 ppm Received ECMO and repeated trials with I-NO Ultimately became septic and died
(23)	7 MAS 1 CDH 1 RDS 1 sepsis 1 coarctation of the aorta	11 infants (2-469 hrs old)	20-80 ppm	7/11 increased PaO_2 1 received ECMO 2 died
(40)	3 PPHN 2 sepsis 1 CDH 1 chronic lung disease with RSV	6 infants during transport	<20 ppm	4/6 recovered "Marked improvement in oxygenation occurred with the onset of NO inhalation"
(41)	PPHN Sepsis	1 premature neonate	20 ppm	Sustained improvement in oxygenation No difference in effect of 10 and 20 ppm on oxygenation Infant ultimately weaned successfully
(42)	3 CDH 4 MAS 3 ARDS, Sepsis	10 term and near-term infants with hypoxemic respiratory failure	8 -80 ppm	4 subjects died 3 received ECMO 3 received I-NO during HFOV No bleeding complications in any subject 3 subjects developed bronchopulmonary dysplasia. All had received I-NO for more than two weeks
(43)	Oligohydramnios Premature rupture of membranes	8 premature infants (24 to 31 weeks gestation)		Uniform improvement in PaO_2 3 died 2 infants died after intraventricular hemorrhage >24 after stopping I-NO

The table below summarizes the results of studies using I-NO in neonates with hypoxic respiratory failure with structural heart disease other than patent ductus arteriosus and intra-cardiac shunts at the level of the atria or congenital diaphragmatic hernia. The information extracted from these papers is primarily related to safety the reported safety results.

Table 5.2.2.1b Studies with I-NO in neonates with hypoxic respiratory failure with congenital heart disease or CHD

Study	Population	N*	Dose of I-NO	Results
(44)	Children undergoing repair of total anomalous pulmonary venous connection	9	80 ppm	Subjects had transient increases in pulmonary artery pressure (PAP) when I-NO was discontinued. Increased PAP resolved within 30 minutes without specific tx
(45)	Subjects with PPHN and congenital mitral stenosis	15 children age 1.9 years (4 months to 14.5 years)	80 ppm	No effect of I-NO on systemic BP, left or right atrial pressure, or systemic vascular resistance was found 2 subjects received I-NO for prolonged period (91 hours and 23 hours), with no increased NO_2 levels (mean 1.8 /0.3 ppm).4
(46)		5 infants during surgery 15 treated with PPHN and post-OR	20, 40 and 80 ppm	11/15 had reduction in PAP with I-NO Dose-dependent increase in MetHgb levels up to I-NO 80 ppm 'We found that concentrations of 80 ppm or higher of NO could not be consistently delivered without exceeding 5 ppm of nitrogen dioxide' 'Our experience has been that is no response to inhaled NO has occurred with either 20 or 40 ppm, the patient will not respond to inhaled NO at higher concentrations'
(47)	Children with acute, bilateral lung disease	19 children (mean age 11 years)	10 or 40 ppm	Improved oxygenation and decreased PVR Mortality and duration of therapy similar for 10 and 40 ppm I-NO MetHgb levels higher in the 40 ppm group (means 3.0/0.3 versus 1.6/0.15) 8/19 subjects died, 5 in 10 ppm group, 3 in 40 ppm group
(48)	5 newborns with alveolar capillary dysplasia	Review of 5 cases	up to 100 ppm	All 5 subjects acutely increased PaO_2, but none had sustained response All 5 died, 4 after receiving ECMO
(49)	1 infant with PPHN and alveolar capillary dysplasia	1	up to 80 ppm	Treatment with both I-NO and prostacyclin had a transient effect. Patient ultimately died without ECMO
(50)	8 CDH 1 oligohydramnios	9 neonates	80 ppm	Before ECMO, I-NO did not change PaO_2, oxygen saturation or OI. All subjects required ECMO. After ECMO, I-NO improved PaO_2, oxygen saturation and OI. 3/9 infants died
(51)	4 CDH		5-80 ppm	3 out of 4 transiently improved oxygenation, but all developed tachyphylaxis to the effects of I-NO Metabolites of I-NO, NO_2- and NO_3-, developed in the 3 responding subjects but not after tachyphylaxis developed

The table below summarizes the results of studies reviewing the use of I-NO as well as other topics related to this review.

Table 5.2.2.1c Studies reviewing the use of I-NO and miscellaneous relevant articles

Study	Population	Results and Notes
(52)	Review article	Review of adult use of I-NO
(53)	Review article	Review of use of I-NO in 17 non-neonates
(54)	Review article	Review of I-NO therapy for PPHN, including discussion of potential toxicity: peroxynitrite and NO_2 production. Refers to a patient who developed methemoglobinemia 18.3% after 16 hours of exposure to I-NO
(22)	Review article	Review emphasizing the clinical trials ongoing and clinical data
(55)	Review article	Review emphasizing animal model data
(56)	Review article	Review of pre-clinical and early clinical data
(57)	Subjects with PPHN	Review of I-NO (10-80 ppm) administration in 123 infants NO_2 levels >3 in 4 subjects 24/90 had initial dose (80 ppm) 4/23 subjects with prolonged exposure to I-NO had methemoglobin levels >4.6% Neonates averaged a lower methemoglobin level than infants >30 days old (0.4±0.1 vs. 0.8,0.1%)
(58)	Neonates with hypoxemic respiratory failure	Randomized trial of ECMO vs. medical therapy (no I-NO). ECMO group had 45% decrease in death compared with control
(59)		ECMO trial
(60)		ECMO trial
(61)	Adults with CHF	Reports that abrupt withdrawal of intravenous nitroprusside leads to a significant increase in pulmonary artery pressures, consistent with rebound
(4)	Review article	Review of the use of I-NO, including extensive discussion of potential toxicities

Greater than 300 neonates are reported in the studies referenced above, although none of the trials was double-blinded and placebo-controlled. Overall, an acute effect of I-NO to increase PaO_2 and decrease OI was seen in a portion of all infants studied ranging from 44% (30) to 100% (5). Across all studies, an average of approximately 60% of the infants exposed to I-NO had an acute increase in PaO_2.

An effect of I-NO to reduce the need for ECMO was claimed by some authors (24, 36), although the majority of the published literature is case reports or case series, with no control groups.

5.2.2.1 Efficacy summary from published literature (cont)

Next, the major efficacy claims from the literature will be summarized, grouped according to body system. Note that no primary data was reviewed, and the claims are those of the respective authors. The two published trials which are included in this review will be considered in later sections.

a. Body as a whole

The effect of I-NO on the need for ECMO and overall mortality has been examined by several authors.

ECMO

With the exception of the three trials included in the NDA, all of the published studies suffer from small numbers of subjects enrolled and/or absence of concurrent, randomized control populations. The absence of an effect of I-NO on the use of ECMO has been reported in one small clinical studies(62). One author estimated that I-NO reduced the need for ECMO by 15% in his uncontrolled trial(24). Another, comparing 21 subjects receiving I-NO to 16 historical controls, asserted that I-NO reduced the need for ECMO from 72 to 25%(36). In this trial, there were 4 deaths in the historical control group, and none in the I-NO group.

Death

In controlled studies, no effect of I-NO to reduce the incidence of death has been reported. In a trial comparing 21 subjects receiving I-NO to 16 historical controls, there were 4 deaths in the historical control group, and none in the I-NO group(36).

b. Cardiovascular system.

An effect of I-NO to reverse the right-to-left intra-cardiac shunt seen as part of pulmonary hypertension was reported by several authors(38, 42, 43).

c. Gastrointestinal system

No specific efficacy claims in this body system were identified in the literature surveyed.

d. Endocrine system

No specific efficacy claims in this body system were identified in the literature surveyed.

e. Hemic and lymphatic system

No specific efficacy claims in this body system were identified in the literature surveyed.

f. Metabolic and nutritional system

No specific efficacy claims in this body system were identified in the literature surveyed.

g. Dermatological system

No specific efficacy claims in this body system were identified in the literature surveyed.

h. Nervous system

No specific efficacy claims in this body system were identified in the literature surveyed.

i. Respiratory system

An effect of I-NO to improve measures of oxygenation has been reported by numerous authors(6, 12, 25, 27, 29, 31, 34, 35, 47). In most, but not all studies, the effect is acute (within one hour). Some infants who improve only after prolonged exposure (>1 hour) to I-NO. It was also reported that this improvement was transient in some infants(24, 31, 39). In other infants, the effect was sustained for periods of at least 24 hours(5, 26).

The dose-dependency of this effect has been examined in only one paper(34). In it, no difference in effect on oxygenation for doses of I-NO from 5 to 80 ppm was detected. Several authors have reported significant improvements in oxygenation using ≤10 ppm I-NO(6, 12, 28, 29, 34, 42). One author reported that I-NO had no effect on oxygenation below 80 ppm(6).

An effect of I-NO to improve intra-pulmonary ventilation-perfusion matching in infants with hypoxic respiratory failure(38). This effect was most prominent in infants with pre-existing right-to-left shunting of blood, and occurred at a median dose of 20 ppm I-NO.

j. Special senses

No specific efficacy claims in this body system were identified in the literature surveyed.

k. Genitourinary system

No specific efficacy claims in this body system were identified in the literature surveyed.

5.2.2.2 Safety summary from published literature

A variety of potential toxicities of I-NO have been postulated in the literature. Additionally, a small number of subjects have had reported toxicities following the administration of I-NO. In this section, these toxicities will be grouped according to body system, sub-divided into acute and chronic toxicities.

a. Body as a whole

Acute Toxicities

None identified

Chronic Toxicities

1. Inhibition of endogenous I-NO production

This potential effect of chronic exogenous I-NO follows the observation that such an exposure leads to the down-regulation of nitric oxide synthase activity(63). It is also similar to the pattern of 'tolerance' that has been observed with other oral and topical nitrates(13, 14). In neonates, who frequently have impaired surfactant production, this might enhance recovery from pulmonary injury.

2. Nitrosylation of proteins

I-NO can interact with the sulfur moieties in proteins, forming S-nitrosothiol groups. These reactive groups can then recombine to alter protein structure and function(4). One consequence of this effect of I-NO may be the inactivation of surfactant, as was recently proposed in neonates exposed to I-NO(50).

3. Carcinogenicity/teratogenicity

This concern is dealt with in section 4.1 above and in the Pharmacologist's review.

b. Cardiovascular system

Acute Toxicities

1. Decreased vascular resistance

In neonates undergoing repair of congenital cardiac anomalies, with left ventricular failure, no changes in atrial pressures were noted following I-NO, 80 ppm. The systemic BP and systemic vascular resistance, however, decreased 7% from baseline(45).

There have rare case reports of precipitous declines in systemic BP following the initiation of I-NO, which was reversed after withdrawal of I-NO(24, 33).

2. Decreased myocardial contractility, especially in settings of poor left ventricular function.

In adults with heart failure, the adminstration of I-NO has been reported to cause an increase in pulmonary capillary wedge pressures and an increase in left ventricular end-diastolic pressure(64). Because of this, the authors recommended caution in using I-NO in the presence of heart failure.

Chronic Toxicities

None identified.

c. Gastrointestinal system

Acute and Chronic Toxicities

1. None identified (increased GI bleeding is considered under Hemic & Lymphatic System).

d. Endocrine system

Acute and Chronic Toxicities

1. None identified

e. Hemic and lymphatic system

Acute Toxicities

1. Bleeding and platelet-dysfunction

A prolongation of bleeding times in normal volunteers was reported in 6 volunteers breathing I-NO, 30 ppm, for 15 minutes. This effect was not seen at I-NO 10 ppm(65). No effect of I-NO on bleeding time was reported in anther study in abstract form(66). These studies were conducted in healthy volunteers. No data on bleeding in neonates and I-NO is available. No increased bleeding has been reported in the literature(24, 33, 42), and no changes in coagulation parameters, where measured (31).

5.2.2.2 Safety summary from published literature (cont)

2. Non-functional hemoglobins/ Methemoglobinemia

Hemoglobin combines with I-NO to form nitrosyl-hemoglobin and then methemoglobin, which is converted back into hemoglobin by the Methemoglobin reductase. Three factors pre-dispose infants to higher methemoglobin concentrations(67).

1) Neonates have diminished activity of methemoglobin reductase;

2) Fetal hemoglobins are also more susceptible to the formation of methemoglobin; and

3) Neonates have a higher gastric pH, due to incompletely developed ability to secrete acid. This permits the growth of nitrate-reducing bacteria in the gastrointestinal tract, increasing the production of nitrites for absorption into the circulation, enhancing methemoglobin production.

Methemoglobin does not carry oxygen, so RBCs containing methemoglobin do not deliver O_2 to tissues. In the hypoxic newborn, this is an obvious safety concern. Several authors have reported elevated methemoglobin levels in neonates exposed to I-NO(35, 57).

3. Increased nitrogen dioxide (NO_2) concentrations

NO_2 forms quickly in gas mixtures containing NO and O_2. NO_2 causes airway inflammation and damage. It has also been proposed that 80-90% of inhaled NO_2 is retained in the lung, exacerbating its potential toxicity(68). Increased NO_2 concentrations have been reported following I-NO therapy, especially 80 ppm(69).

Chronic Toxicities

1. Increased red blood cell turnover

In mice, exposure to I-NO, 10 ppm, for 6 months caused increased RBC turnover and increased spleen weight(70). No known human studies on this effect have been performed.

f. Metabolic/Nutritional system

Acute and Chronic Toxicities

None identified

g. Nervous system

Acute and Chronic Toxicities

None identified

h. Respiratory system

Acute Toxicities

1. Pulmonary inflammation due to NO_2 production.

As discussed above, exposure to I-NO can lead to NO_2 production.

Acute toxic pulmonary effects that have been reported in animals at or below 5 ppm NO_2 include: altered pulmonary surfactant production and diffuse inflammation(19, 71); epithelial hyperplasia of the terminal bronchioles and pulmonary hyper-reactivity in rats(72). In rats, histologic injury to the lung has been reported after 30 minutes of exposure to NO_2, 25 ppm, but not at lower doses. There was no significant increase in histologic evidence of lung injury after up to 30 minutes of exposure to 10 or 25 ppm NO_2(73). Higher doses than NO_2 5 ppm chronically cause pulmonary edema and hypoxic death in animals.

The lower level of safety for NO_2 exposure in humans is difficult to establish, as NO_2 levels as low as 0.5 ppm have been reported to enhance human airway reactivity(74, 75). An acute decrease of 40% in effective lung capacity, along with a 92% increase in viscous resistance, has been reported in healthy volunteers following a 10 minute exposure to 4-5 ppm NO_2. Volunteers exposed to 5 ppm NO_2 for 15 minutes also had a decreased CO_2 diffusing capacity. Whether NO_2 has a greater or lesser toxicity in neonates with underlying severe pulmonary injury is unknown.

The U.S. National Institute for Occupational Safety and Health recommends a time-weighted exposure of 5 ppm NO_2 over 8 hours(76). This is a higher level than is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH), which is 3 ppm per 8 hours with a strict ceiling of 5 ppm NO_2 (15 minute exposure). Ohmeda proposes to use the NIOSH standard.

Chronic Toxicities

1. Increased pulmonary artery pressures after discontinuation of I-NO

The rapid withdrawal of I-NO has been reported cause a rebound pulmonary hypertension and an abrupt fall in PaO_2, This has been reported in children who have undergone surgery for repair of congenital cardiac lesions(44, 45)]. It has also been reported in children with hypoxemic respiratory failure and no congenital cardiac lesions(37). This effect has been linked with the decreased endogenous NO production following chronic exposure to exogenous I-NO.

5.2.2.2 Safety summary from published literature (cont)

2. Altered pulmonary surfactant production

Altered surfactant production has been reported rats exposed to 100 ppm I-NO for 24 hours. Whether this is linked to possible effect of NO_2 is not known. An effect of I-NO to inhibit surfactant has been suggested in human neonates(50).

2. Peroxy-nitrile-induced lung injury

Inhaled O_2 and I-NO are co-administered in these children, these two gases may potentiate each other's toxic effects. Exposure to high concentrations of O_2, as in the present trials, can lead to increased superoxide formation in the lung, which in turn stimulates the formation of peroxy-nitrite from I-NO(54, 77). These oxidative species can interact with pulmonary proteins, including catalase and surfactant, to induce lung injury.

In one study, 3 of 10 subjects exposed to I-NO up to 80 ppm developed bronchopulmonary dysplasia(42).

i. Special senses

Acute and Chronic Toxicities

None identified

j. Genitourinary system

Acute and Chronic Toxicities

None identified

5.2.2.3.Efficacy of ECMO in hypoxic respiratory failure

In the trials below, avoidance of ECMO was a primary endpoint in the largest completed trial (NINOS). This section reviews the use of ECMO in the treatment of hypoxemic respiratory failure in neonates, and the data supporting the utility of ECMO in this population.

ECMO is performed by removing the infants blood, oxygenating and warming it, and then returning back to the infant. This has historically been performed using the venoarterial bypass, where blood is removed from the right internal jugular, oxygenated, and then returned via the right common carotid artery. Both the jugular vein and carotid artery are usually ligated after the procedure, although surgical repair is performed, especially of the carotid artery. More recently, veno-venous ECMO has been proposed, which may make ECMO less invasive, and spare the carotid artery. The two modalities appear to be of equal clinical efficacy. ECMO also requires systemic heparinization, with an attendant increase in the risk of bleeding, especially intracranial hemorrhage. ECMO also requires a large commitment in hospital resources, and is limited to large hospitals. Infants are frequently transported to receive it, with the attendant risks of such transport in critically ill infants.

There have been three major clinical trials that have examined the clinical utility of ECMO, of which the UK collaborative trial was both blinded and randomized for all infants (58-60, 78).

In the UK collaborative randomized trial of neonatal ECMO, 185 near-term infants with severe hypoxic respiratory failure were randomized to either referral to a specialist center for consideration of ECMO, or continued intensive conventional management at the original hospital. The trial was stopped after 185 infants were enrolled the data accumulated showed a clear advantage towards the group referred for ECMO. Twenty-eight of 93 subjects referred for consideration of ECMO died (30%), compared with 54 of 92 subjects allocated to conventional therapy (59%), corresponding to a 45% reduction in the mortality rate. The relative risk for death in the two groups favored the ECMO group (0.55 with 95% confidence interval 0.39 to 0.77, p=0.0005). This difference in survival persisted irrespective of the primary diagnosis, disease severity, and referral center.

Less data exists for the long-term efficacy of ECMO, although approximately 70% of the infants who received ECMO in the late 1980's have been reported to have normal developmental scores 1-4 years after discharge(79).

5.3 Comment on Adequacy of Clinical Experience

The clinical experience that forms the basis of this NDA comes from four trials, of which two were completed. A total of 278 subjects were exposed to I-NO at doses ranging from 5-100 ppm. This includes 7 subjects in the NINOS trial who were randomized to receive control gas but instead received I-NO. Of the subjects who received I-NO as part of the NDA submission, 264 received I-NO as part of a double-blinded, randomized study. The remainder, 14 subjects, received I-NO as part of an open-label safety study.

5.4 Comment on Data Quality and Completeness

The adequacy of the database will be reviewed by examining the data submitted for each of the four trials in turn. Specific deficiencies will also be commented on in each individual trial. See Table 5.1.1 for a summary of the safety data collected for each trial.

5.4.1 Data quality and completeness in the NINOS trial

The NINOS trial was planned and conducted under academic auspices. Only selected laboratory data were submitted regarding the endpoints of the trial. For example, the individual PaO_2 and OI values for all of the infants throughout the trial were not collated, although the PaO_2 and OI values at baseline and 30 minutes after treatment with study gas were collected and submitted. Similarly, no laboratory data on routine serum chemistries, hematology or bleeding parameters were collected.

The NINOS trial also did not collect case report forms in the format normally seen of a pivotal trial. These would normally include copies of all of the lab data, vital signs, and drugs administered throughout the hospitalization for each subject, along with any adverse events. Instead, the summary data-sheets which were submitted to the statistical center were also submitted to the FDA. These contain the results of certain key measurements made during the trial, and selected relevant treatments given to the infant. For example, the presence or absence of interventricular hemorrhage was noted, but the original notes concerning the extent of that IVH (from the radiologists or attending neonatologists) were not included.

During the NINOS trial, data on selected adverse events was collected (e.g., IVH, seizures, death). No information regarding the unanticipated adverse events or abnormal laboratory values is available.

The long-term follow-up data for the NINOS trial has not yet been collected for the majority of the surviving infants. None of the data from these subjects has been submitted to the FDA.

With those caveats, the short-term data (0-28 days, or until discharge or death) were submitted in both paper form and in SAS datasets.

5.4.2 Data quality and completeness in the INOSG trial

The INOSG trial has several critical deficiencies which limit its use beyond the acute (20 minute) time-point. First, the INOSG trial was planned and conducted under academic auspices, and safety data was collected only for those adverse events that were judged to be important by the investigators. This includes methemoglobin levels, but not NO_2 levels, for instance. Methemoglobin levels for the responding subjects who received I-NO are the only lab values submitted for the trial. Data on overall adverse events, including adverse events which altered therapy, were not collected.

A second deficiency is the lack of primary efficacy data. No individual patient data for the control subjects after 20 minutes of therapy with study gas were submitted. Instead, the sponsor submitted long-term data only for those subjects who received and responded to I-NO. This limits the analysis of any effect of I-NO to acute events (up to 20 minutes) and the primary endpoint (acute improvement in oxygenation. Since the investigators were able to separate (and selectively follow) those subjects who received I-NO and responded from the responding subjects who did not receive I-NO, and from the non-responding subject who received I-NO, the maintenance of the blinding must also be called into question. These issues will be discussed further in the review of INOSG.

A third deficiency is the lack of case reports for any of the infants in the study. At the reviewer's request, the sponsor submitted summaries for the 5 deaths.

With the caveats listed above, the data were submitted in both paper form and in SAS datasets.

5.4.3 Data quality and completeness in the INO-01/ -02 and INO-03 trials

The INO-01/ -02 and INO-03 trials were performed under the auspices of Ohmeda. Both trials collected and submitted original data on all subjects throughout their hospitalization. Per protocol, however, the data submitted varied in their completeness. Data directly relevant to the endpoints of the trial (e.g., all measured PaO_2 values, ventilator settings) and major outcomes (adverse events identified by the investigators, death ECMO) were collected and submitted in full. For details of the adverse event data collected, see section 8.5.1.1 below. However, data regarding unanticipated adverse events, especially lab values, were submitted only for selected time points. For details of the lab values submitted, see section 8.1.6 below.

Case Report Forms (CRFs) were submitted for all serious adverse events, trial drop-outs, and deaths. Other CRFs were submitted for individual subjects at the request of this reviewer. These CRFs detail the changes in oxygenation and ventilation, as well as the medications and measured methemoglobin and NO_2 levels. No additional follow-up information was available for identified adverse events from these CRFs.

The data for the INO-01/ -02 and INO-03 trials were submitted in both paper form and in SAS datasets.

6.0 Review of Individual Studies

The next four sections of this review will, in turn, examine each of the trials submitted as part of the NDA.

6.0.1 NINOS Study

6.0.1.1 Title of Study: Neonatal Inhaled Nitric Oxide Study (NINOS)

6.0.1.2 Site(s) of Investigation and Investigators:

The NINOS study sites and investigators are listed in table 6.0.1.2.1 below.

Table 6.0.1.2.1 NINOS study sites and investigators.

NICHD Neonatal Research Network Clinics			Canadian Inhaled Nitric Oxide Study Group	
Site	Investigators		Site	Investigators
National Institute of Child & Human Development (NICHD)	L. Wright, M.D. (Project Manager)		University of Alberta Royal Alexander Hospital	N. Finer, M.D. A. Peliowski, M.D.
George Washington University Biostatisitics Coordinating Center	J. Verter, Ph.D N. Younes, Ph.D.		University of Alberta University of Alberta Hospital	K. Barrington, M.D.
Yale University Yale-New Haven Hospital	R. Ehrenkranz, M.D. (Principle Investigator)		University of British Columbia British Columbia Children's Hospital	A. Solimano, M.D.
Case Western Reserve University Rainbow Babies and Children's Hospital	E. Stork, M.D. A. Fanaroff, M.D.		University of Calgary Foothills Hospital	N. Singhal, M.D.
Wayne State University Children's Hospital of Michigan	G. Konduri, M.D. S. Shankaran, M.D.		McMaster University Chedoke-McMaster Hospital	H. Kirpalani, M.D.
University of Tennessee, Memphis E.H. Crump Hospital	D. Crouse, M.D., Ph.D. S. Korones, M.D.		University of Ottawa Children's Hospital of Eastern Ontario	R. Walker, M.D.
University of New Mexico University of New Mexico Medical Center	M. Crowley, M.D. L. Papile, M.D.		McGill University Montreal Children's Hospital	A. Johnson, M.D.
University of Cincinnati University of Cincinnati Hospital	R. Brilli, M.D. E. Donovan, M.D.		University of Saskatoon Royal University Hospital	H. Kirpalani, M.D.
Indiana University Indiana University Hospital	G. Sokol, M.D. J. Lemons, M.D.		Universite De Sherbrooke Center Hospitalier Universitaire	P. Blanchard, M.D.
Brown University Women and Infants Hospital	R. Rothstein, M.D. W. Oh, M.D.		University of Manitoba Women's Hospital Health Sciences Center	C. Fajardo, M.D.
Stanford University Packard Children's Hospital	K. Van Meurs, M.D. W. Rhine, M.D. D. Stevenson, M.D.		Baylor University Texas Children's Hospital	M. Gomez, M.D.

6.0.1.3 Background

5.2.94: NINOS first submitted to the FDA in the form of a revised draft.
12.1.94: Revised protocol, used during the initiation of NINOS.
1.26.95: Further revisions of NINOS approved by the NINOS Executive Committee in a letter dated 3.3.95
5.11.95: Further revisions of NINOS approved by the NINOS Executive Committee in a letter dated 7.12.95.
5.2.96: NINOS trial was halted at the recommendation of the trial's Data Safety and Monitoring Board.
2.27.97: The results of the NINOS study were published(1).
3.31.97: NINOS trial results were submitted to the FDA.

6.0.1.4 Study Design

The NINOS trial was a multi-center, multi-national, double-blind, placebo-controlled trial designed ...'to evaluate the efficacy of I-NO (inhaled nitric oxide) in the treatment of term and near-term infants with hypoxic respiratory failure.'

Subjects with hypoxic respiratory failure (see inclusion and exclusion criteria) were randomized to one of two groups:

1) a control group who received either oxygen (or no flow of I-NO);
2) a treatment group who received I-NO for up to 336 hours (14 days). Initially, subjects received I-NO 20 ppm.

After 30 minutes on study gas ('low-flow'), the subject's response was measured:

Full response: > 20 mmHg increase in PaO_2.
Partial response: 10-20 mmHg increase in PaO_2.
No response: <10 mmHg increase in PaO_2.

6.0.1.4 Study Design (cont)

Subjects who responded fully were continued on the 'low-flow' study gas (either placebo (O_2) or I-NO, 20 ppm).

Subjects who had no response, or responded partially, were entered into the 'high-flow gas' protocol. These subjects were administered either placebo gas (O_2) or to I-NO, 80 ppm, depending on their initial randomization. Their response was measured after another 30 minutes, and based on the above criteria for successful response, the high-flow gas was either continued or discontinued.

Non-responders to the high-flow gas were weaned off of the study gas. They were eligible for a repeat trial of the same study gas (either low- or high-flow) after 6 hours, so long as the infant was still otherwise eligible. This process could be repeated three times. If no positive response was observed after 3 repeat trials (a total of 4 trials), the subject was labeled a non-responder.

Responders to high-flow study gas (I-NO 80 ppm or O_2 placebo) underwent regularly scheduled weaning attempts every 2 hours for the first 12 hours, and then every 12±2 hours. Weaning attempts continued until either the gas was completely discontinued, the weaning attempt was unsuccessful or the maximal exposure to study gas was reached.

Responders to low-flow study gas (I-NO 20 ppm or O_2 placebo) also underwent regularly scheduled weaning attempts every 12±2 hours. Weaning attempts continued until either the gas was completely discontinued, the weaning attempt was unsuccessful, or the maximal exposure to study gas was reached.

After 240 hours of gas administration, the study gas concentration had to have been ≤5 ppm I-NO or equivalent O_2 flow. Maximum exposure to study gas administration was to 336 hours (14 days).

Both groups received all other conventional therapies for hypoxic respiratory failure, including surfactant and high frequency jet ventilation. Each center's guidelines determined the mode of ventilation, the use of tolazoline and therapies to maintain arterial pressure, induce alkalosis, and provide sedation/ analgesia. The mode of ventilation was not to be changed after randomization, other than during weaning or ventilatory support. Additionally, the use of surfactant was restricted: the subject could receive surfactant only if it was initiated prior to randomization. Subjects transferred to ECMO could continue on study gas if the masking could be maintained. The infant was to receive the same study gas he/she received prior to the transfer.

Subjects were eligible for transfer to ECMO if they met the criteria set forth in the dosage/administration section below. The decision to initiate ECMO therapy was ultimately made by the individual investigator, and not all infants who met the eligibility criteria for ECMO received it.

Infants, including those who received to ECMO, were followed for adverse events until death, discharge to home, or 120 days. Survivors were also followed after discharge, for a planned analysis of neurodevelopmental outcomes to be performed 18-24 months after initial study gas administration.

6.0.1.5 Primary and Secondary Endpoints

Primary Endpoint

1. Incidence of death before discharge or 120 days (whichever comes first), and/or the initiation of ECMO

A significant benefit was defined as a >40% relative reduction in the risk of death or of the initiation of ECMO in the I-NO-treated group, relative to the control group (NDA volume 2.35, page 005310).

Secondary Endpoints

1. Change in PaO_2 levels measured 30 minutes after initial administration of the study gas.
2. Change in mean OI levels measured 30 minutes after initial administration of the study gas.
3. Change in Aa-DO_2 levels before and 30 minutes after initial administration of the study gas.
4. Neurodevelopmental outcomes assessed at 18-24 months corrected age.
5. The average length of hospitalization among surviving infants.
6. The number of days of assisted ventilation.
7. The incidence of air leak.
8. The incidence of chronic lung disease.
9. The proportion of infants transferred for potential ECMO.

6.0.1.6 Number of Subjects/ Randomization

A total of 250 subjects were planned for enrollment. A total of 235 enrolled: 121 subjects in the control group and 114 in the I-NO group.

The table below summarizes the enrollment in NINOS by site.

Table 6.0.1.6.1 Enrollment in NINOS by site.

Site	Patients Enrolled
Case Western Reserve University	14
Wayne State University	28
University of Tennessee, E.H. Crump Hospital	2
University of New Mexico	5
University of Cincinnati Hospital	7
Indiana University Hospital	21
Brown University, Women and Infants Hospital	3
Stanford University, Packard Children's Hospital	28
University of Alberta; Royal Alexander Hospital	21
University of Calgary, Foothills Hospital	15
University of Alberta Hospital	2
University of British Columbia	15
Baylor Hospital, Texas Children's Hospital	31
McGill University Montreal Children's Hospital	16
University of Ottowa	3
McMaster University, Chedoke-McMaster Hospital.	16
University of Manitoba	8
Royal University Hospital	3
Sherbrooke University	1
Total	235

Randomization

Two teams were used to accomplish the blinding in the trial. The first team consisted of the patient caregivers, who were blinded to the treatment gas being administered. The second team consisted of a least one unblinded investigator, who was responsible for all activities that revealed the treatment gas. These activities included maintenance of the bedside stock of treatment gas, daily calibration of the gas blender, and recording the methemoglobin, I-NO, and NO_2 levels.

The time from randomization to the start of treatment gas was 45±238 hours in the control group and 29.3±39 hours in the I-NO group. This difference was not statistically significant.

6.0.1.7 Inclusion/Exclusion Criteria

Inclusion Criteria

1. The infant must require mechanical ventilation for hypoxic respiratory failure.
2. A diagnosis of one of the following: PPHN; respiratory distress syndrome; meconium aspiration syndrome; pneumonia/sepsis or suspected pulmonary hypoplasia. Echocardiographic evidence of pulmonary hypertension was not required.
3. The infant must have an indwelling arterial line.
4. The infant must have an Oxygenation Index (OI) ≥25 on two arterial blood gases.
5. The infant must have echocardiographic evaluation preferably within the 24 hours preceding randomization.
6. Appropriate consent must be given.

Exclusion Criteria

1. A gestational age ≤34 weeks.
2. An age after birth of >14 days.
3. A decision not to provide full treatment (e.g. in the case of chromosomal abnormalities or severe birth asphyxia).
4. Known structural congenital heart disease, other than patent ductus arteriosus and intra-cardiac shunts at the level of the atria.
5. Enrollment in a conflicting clinical trial.
6. Opposition to enrollment from the attending neonatal physician.

6.0.1.8 Dosage/Administration

Subjects are randomized to receive either I-NO (20 ppm) or control gas (O_2) based on the inclusion/exclusion listed above.

After 240 hours of gas administration, the study gas concentration had to have been ≤5 ppm I-NO or equivalent O_2 flow. Total study gas administration was limited to 336 hours (14 days).

Because of the hazards associated with potentially high NO_2 levels and the potential for methemoglobinemia, it was decided that an unmasked investigator would monitor these parameters during the trial. To maintain a double-blind study, therefore, it was necessary to establish two clinical teams. The unmasked investigators were responsible for all activities and data collection that could potentially indicate which treatment gas was being administered. All other personnel caring for the infant remained masked as to the treatment gas identity.

I-NO was delivered in a concentration of 800 ppm (from or BOC Gases America). The source gas was delivered to the subject using a flow meter or an equivalent) and an approved neonatal ventilator (Infant Star or Sechrist IV 100B). The high flow of study gas (used to minimize the dwell time of the I-NO with O_2 in the lines) meant that the maximum FiO_2 for subjects receiving I-NO was 90%, less than the 95-100% FiO_2 they received prior to starting I-NO. The subjects who were randomized to receive placebo or control gas were administered O_2 at flow rates corresponding to the I-NO.

6.0.1.9 Duration/Adjustment of Study

Subjects continued in the study, subject to the following sets of criteria:

Eligibility criteria for increase in study gas

1. If an infant failed to respond to initiation of study gas therapy with an increase in PaO_2 of at least 20 mmHg after 30 minutes, study gas was to be discontinued. After 15 minutes, another blood gas was obtained, and the infant randomized to either I-NO 80 ppm or high-flow placebo.

2. If an infant's respiratory status worsened while on study gas therapy, the gas concentration was doubled unless the current dose was already high-flow (80ppm I-NO vs. placebo gas). Deterioration was defined as aworsening of two OIs at least 30 minutes apart if both were ≥25 and both were >50% worse than the OI measured at time of last weaning attempt.

Eligibility criteria for re-trial with study gas after initial failure to respond

If an infant failed to respond to the study gas at both low- and high-flow rates, up to three additional trials of study gas could be tried at intervals of at least 6 hours if they met the following criteria:

1. The infant must still require assisted ventilation for hypoxic respiratory failure.
2. The infant must still have an OI ≥25, obtained at least 6 hours after last use of I-NO.
3. No decision has been made to limit therapy.
4. The infant has not enrolled in a conflicting trial.
5. The attending physician supports the re-trial.
6. I-NO has been administered to the subject no more then three times, not counting the initial administration.

Eligibility criteria for re-initiation of study gas therapy

If an infant is successfully weaned from study gas, but subsequently declines, they may be placed on study gas again, if the following criteria are met:

1. The infant must still require assisted ventilation, although not necessarily for hypoxic respiratory failure.
2. The infant must have an OI≥15 on two arterial blood gases at least 30 minutes apart.
3. I-NO was re-initiated at the equivalent of I-NO 5 ppm ("very low flow study gas").

a. If the subject had an increase in PaO_2 >20 mmHg 15-30 minutes after initiation, the gas was continued at that rate. Weaning was attempted as detailed above.

b. If there was not a positive response to very low flow study gas, the study gas was increased to 20 ppm ("low flow study gas"). Subsequent management was as detailed above for subjects receiving low-flow gas.

Criteria for transfer of subject to ECMO

An infant could be transferred to ECMO for the following clinical indications:

1. OI>40 on two ABGs separated by at least 30 minutes.
   or
   OI>35 for 4 hours.

2. A-aDO_2 >630 for 4 continuous hours.
   or
   A-aDO_2 >620 for 12 continuous hours.

6.0.1.9 Duration/Adjustment of Study (cont)

3. Acute deterioration/unresponsiveness to medical therapy (any 2 of 4):
    a. PaO_2 <55 mmHg for at least 2 hours.
    b. pH <7.15, or <7.4 if alkalosis attempted for at least 2 hours.
    c. Mean blood pressure <40 mmHg for at least 2 hours.
    d. Severe barotrauma (at least 4 of the following 7 criteria):
        1. Pulmonary interstitial emphysema/pseudocyst
        2. Pneumothorax/pneumomediastinum
        3. Pneumoperitoneum
        4. Pneumopericardium
        5. Subcutaneous emphysema
        6. Persistent air leak >24 hours
        7. Mean Airway Pressure (MAP) >15 cm H_2O

While these criteria were used to qualify an infant for ECMO, the initiation of ECMO was ultimately based on the clinical assessment of the attending physician. There was no set time established when the assessment for transfer to ECMO was to be performed (personal communication with Dr. Ehrenkranz (NINOS Principle Investigator). This meant that some infants were assessed before being randomized, while others were assessed some time after receiving study gas. The time when the assessment was performed was not recorded.

6.0.1.10 Safety & Efficacy Parameters Measured

Efficacy Parameters

Primary efficacy parameters (per the sponsor)

1. Incidence of neonatal deaths before discharge or 120 days.
2. Incidence of ECMO initiation.

Secondary efficacy parameters

3. Length of stay in hospital.
4. Days on assisted ventilation.
5. Days on continuous positive airway pressure (CPAP).
6. Days on high-dose O_2 (FiO_2 >60%).
7. Days on low-dose O_2 (21% < FiO_2 <60%).
8. Incidence of discharge home on O_2.
9. Oxygenation parameters: PaO_2; % oxygen saturation; postductal PaO_2; preductal O_2 saturation; postductal O_2 saturation; Arterial-alveolar O_2 ratio; Arterial-alveolar O_2 gradient. No pH or pCO_2 data were collected.
10. Hemodynamic parameters: Mean Arterial Pressure; Positive Inspiratory Pressure.

Safety Parameters

1. Methemoglobin levels.
2. Inhaled NO_2 concentrations.
3. Routine lab chemistries and blood counts.
4. Incidence of chronic air leak (pneumothorax, pneumopericardium, pneumoperitoneum, pneumomediastinum, subcutaneous or interstitial emphysema).
5. Incidence of chronic lung disease.
6. Incidence of intracranial abnormalities and risk factors for abnormal neurological sequelae (seizures, intraventricular hemorrhage and brain infarct).
7. Incidence of pulmonary or gastrointestinal hemorrhage.
8. Hemodynamics (heart rate, blood pressure, cardiac output).

6.0.1.11 Statistical considerations

The primary analysis for the primary endpoint was an 'intent-to-treat' analysis. All infants randomized were assessed for the primary outcome (death before discharge or 120 days and/or the initiation of ECMO). Data for all infants were analyzed by the treatment group to which they were randomized, regardless of treatment received.

Additional analyses, including summaries of the results in a prior defined groups were performed. The analyses for secondary and exploratory hypotheses were based on the appropriate statistical tests depending on the type of outcome (e.g.; analysis of covariance, two sample t- or Wilcoxon tests).' (from NDA volume 2.14, page 024408).

Two interim analyses were proposed, after 1/3 and 2/3 of the subjects were enrolled. 'In order to minimally maintain the overall type I error for the trial at 0.05, the level defined for 'statistical significance' at each of the analyses was adjusted using the group-sequential method of Lan and DeMets with the modified O'Brien-Fleming spending function.

6.0.1.12 Efficacy endpoint outcomes

6.0.1.12.1 Patient demographics and baseline characteristics

The first table shows the baseline demographics of the randomized NINOS subjects. The two groups were well-matched with regards to their demographics.

Table 6.0.1.12.1.1 Demographics of NINOS subjects[a].

Variable	Control	I-NO	p-value[a]
Total # with evaluable data	119/121	111/114	
Sex			
Male	76 (63%)	63 (55%)	0.24
Race			
White	72 (61%)	70 (63%)	
Black	19 (16%)	19 (17%)	
Hispanic	17 (14%)	13 (12%)	
Other	11 (9%)	9 (8%)	
Missing	2 (<1%)	3 (<1%)	
Age at start of study gas (days)	1.7±2.3	1.7±1.8	0.78
Birth Weight (kg)	3.4±0.6	3.5±0.6	0.19
Gestational Age (weeks)	38.9±2.2	39.3±1.8	0.16

a. Presented as # of subjects and % of subjects with evaluable data. p value calculated using chi-square test.

The next table shows the underlying causes of hypoxic respiratory failure for the randomized NINOS subjects. The two groups were well-matched with regards to their underlying disease state.

Table 6.0.1.12.1.2 Underlying disease of subjects enrolled in NINOS.

Disease	Control	Inhaled I-NO
Meconium Aspiration (% of total)	58 (48%)	58 (51%)
Idiopathic PPHN	22 (18%)	19 (17%)
Sepsis/pneumonia	24 (20%	26 (23%)
Respiratory Distress Syndrome	15 (12%)	10 (9%)
Suspected Pulmonary Hypoplasia	1 (1%)	1 (1%)
Other	1 (1%)	0 (0%)

Almost all subjects had echocardiograms (97% of placebo subjects, 98% of I-NO subjects). The results, which show that the echocardiographic characteristics of the two groups were well-balanced, are summarized below. Note that a significant percentage of the subjects in the NINOS had evidence of left to right shunt on ECHO (hence, did not have pulmonary hypertension severe enough to cause reversal of normal pressure gradient). These individuals would not have been accepted into the INO-01/-02 or INOSG trials, as they did not meet the echocardiographic definition of PPHN.

6.0.1.12.1 Patient demographics and baseline characteristics (cont)

Table 6.0.1.12.1.3 Echocardiographic characteristics of randomized subjects.

	Placebo Group (n=121)	I-NO Group (n=114)	p value[a]
Echocardiogram performed	118/121 (98%)	110/114 (97%)	0.64
Patent ductus arteriosus (PDA) identified	92/118 (78%)	88/110 (80%)	0.71
Direction of PDA shunt			
Right to left	19/92 (21%)	18/87 (21%)	0.82
Bidirectional	43/92 (47%)	37/87 (42%)	
Left to right	30/92 (32%)	32/87 (37%)	
Tricuspid regurgitation identified[b]	76/117 (65%)	63/109 (58%)	0.27
Foramen ovale (FO) shunt identified[b]	99/117 (85%)	81/108 (75%)	0.07
Direction of FO shunt[b]			
Right to left	23/99 (23%)	21/78 (27%)	0.6
Bidirectional	39/99 (39%0	25/78 (32%)	
Left to right	37/99 (37%)	32/78 (41%)	
Right ventricular function[b]			
Normal	79/110 (72%)	71/104 (68%0	0.85
Decreased	29/110 (26%)	31/104 (30%)	
'Markedly" decreased'	2/110 (2%)	2/104 (2%)	
Interventricular septum flattening[b]	45/112 (40%)	47/106 (44%)	0.53
Local diagnosis of PPHN[b]	82/117 (70%)	60/110 (63%)	0.24
Clinical diagnosis of PPHN[b]	95/117 (81%)	81/109 (74%)	0.21

a. p value calculated using chi-square or Student's t-test, as appropriate (see NDA, vol. 2.35, page 009510.
b. data shown as % of subject with available data.

The next table summarizes the birth characteristics and baseline clinical parameters, which were similar in the two groups. The average OI is quite high in both groups, identifying an extremely 'sick' population. Compare this with the baseline OI in the INO-01/ -02 trial of approximately 25 and in the INOSG trial of 42-45.

Table 6.0.1.12.1.4 Baseline clinical parameters of subjects enrolled in NINOS[c].

Clinical Characteristic	Control	Inhaled I-NO	p-value[a]
Birth characteristics			
Cesarean section	57 (47%)	59 (52%)	0.48
Apgar at 1 minute	5.5±2.7	5.2±2.8	0.36
# with 1 minute Apgar <3	25 (21%)	28 (25%)	0.47
Apgar at 5 minutes	7.2±2.2	7.1±2.1	0.78
# with 5 minute Apgar <3	4 (3%)	5 (4%)	0.69
Pulmonary status			
Oxygenation Index #1[b] (OI) (cm H_2O/mmHg)	45±22	43±17.6	0.46
Oxygenation Index #2 (OI)	46.3±19.9	47.3±31.3	0.76
Oxygenation Index #3 (OI)	43.1±25.5	39.8±24.6	0.43
PaO_2 (first determination) (mmHg)	45.5±13.9	46.8±15.5	0.51
A-aDO_2	615±38	614±36	0.81
Mean Airway Pressure (cm H_2O)	19±4.9	18.7±4.1	0.6

a. p value calculated using chi-square or Student's t-test, as appropriate (see NDA, vol. 2.35, page 009510.
b. Up to three OI reading could be taken prior to initiation of study gas. A majority of both groups received three OI readings (90% in placebo, 84% in I-NO group).
c. No hemodynamic data (blood pressure, heart rate) prior to randomization is available.

6.0.1.12.1 Patient demographics and baseline characteristics (cont)

Other conditions present at baseline were also well-balanced between the two groups. A numerically larger number of subjects in the control group had brain infarcts and/or intraventricular hemorrhage.

Table 6.0.1.12.1.5 Other conditions noted prior to use of study in the NINOS trial[a].

Characteristic	Placebo Group (n=121)	I-NO Group (n=114)	p value[a]
Air leak syndrome	25 (21%)	20 (18%)	0.54
Pulmonary hemorrhage	21 (17%)	18 (16%)	0.75
Prolonged oozing	2 (2%)	6 (5%)	0.13
GI bleeding	1 (1%)	5 (4%)	0.08
Seizures requiring therapy	7 (6%)	12 (11%)	0.18
Brain Infarct	2/88 (2%)	0/82 (0%)	0.17
Interventricular hemorrhage (IVH)[b]	9/87 (10%)	3/82 (4%)	0.10
IVH Grade I	8/9 (89%)	3/3 (100%)	0.10
IVH Grade II	1/9 (11%)	0/3 (0%)	0.55
Periventricular leukomalacia	2/88 (2%)	0/82 (0%)	0.17

a. p value calculated using chi-square or Student's t-test, as appropriate (see NDA, vol. 2.35, page 009510.

b. An equal number of subjects in both treatment groups were evaluated with cranial ultrasound (73%). Thus, the total number of evaluations in the control group was 88/121 (73%) and in the I-NO was 82/114 (73%).

Other therapies received by the subjects prior to randomization were also well-balanced between the two groups.

Table 6.0.1.12.1.6 Specific therapies used by subjects in NINOS prior to randomization.

Specific Therapy	Control	Inhaled I-NO	p-value[a]
Ventilation			
Conventional Mechanical Ventilation	76 (63%)	75 (66%)	0.39
High Frequency Oscillatory Ventilation (HFOV)	42 (35%)	33 (29%)	
High Frequency Jet Ventilation (HFJV)	3 (3%)	4 (4%)	
Specific Therapies:			
Resuscitation required			
Alkalosis therapy[c]	106 (88%)	88 (77%)	0.04
Steroid therapy	20 (17%)	15 (13%)	0.47
Surfactant dose[b]	87(72%)	81 (71%)	0.89
Paralysis/sedation	120 (99%)	113 (99%)	0.45
Tolazoline infusion	17 (14%)	23 (20%)	0.22
Vasopressor support[c]	121 (100%)	108 (95%)	0.01

a. p value calculated using chi-square or Student's t-test, as appropriate (see NDA, vol. 2.35, page 009510).

b. This column reflects the number of subjects who received surfactant at any time prior to randomization. There was also no significant difference between the type (artificial or animal-derived) or amount of surfactant given, or the time when it was given prior to randomization (see NDA vol. 2.35, page 010010-010310).

c. Clinical parameters in which the two groups differed significantly.

6.0.1.12.2 Disposition of subjects

6.0.1.12.2a Subject selection

No information regarding the number of subjects screened versus the number enrolled into NINOS is available. From a personal conversation with the principle investigator of the NINOS trail, Dr. Ehrenkranz, a large fraction (>50%) of subjects who were evaluated were ultimately randomized.

6.0.1.12.2b Protocol Violations and Deviations

The table below list the reported protocol violations and deviations reported by the sponsor.

Table 6.0.1.12.2b.1 Entry criteria violations and protocol violations for NINOS study.

Characteristic	Placebo Group 121 subjects	I-NO Group 114 subjects	p-value
Randomized but did not receive study gas	4 (3%)	1 (1%)	0.20
Randomized but ineligible	3 (2%)	7 (6%)	0.14
Unblinded	3 (2%)	6 (5%)	0.22
Wrong gas given	7 (6%)	1 (1%)	0.04
Received >80 ppm I-NO	0 (0%)	2 (2%)	0.23
Responded to 20 ppm but received 80 ppm I-NO	0 (0%)	2 (2%)	0.23
Received >5 ppm I-NO for>240 hours	0 (0%)	1 (1%)	0.49

6.0.1.12.2c Concomitant therapies used after randomization in the NINOS trial

The subjects in the NINOS trial received a number of other therapies with potential effects on the outcome. The table below compares the therapies received by the two groups, as a % of the subjects with data. No apparent difference exists in the administration of these three types of therapies to the two groups of subjects after randomization.

Table 6.0.1.12.2c.1 Concomitant therapies used after randomization.

Therapy	Placebo Group 121 subjects	I-NO Group 114 subjects	p-value
Ventilation			
High-frequency oscillatory ventilation (HFOV) (%)	39/118 (33%)	46/114 (40%)	0.25
High frequency jet ventilation (HFJV) (%)	3/120 (3%)	4/114 (4%)	0.65
Surfactant			
Any dose	30/120 (25%)	36/114 (32%)	0.26
Standard dose	28/30 (93%)	33/36 (92%)	0.80
Artificial surfactant	1/30 (3%)	0/36 (0%)	0.36
Animal-derived surfactant	29/30 (97%)	35/36 (97%)	
Steroids	28/118 (24%)	35/113 (31%)	0.22

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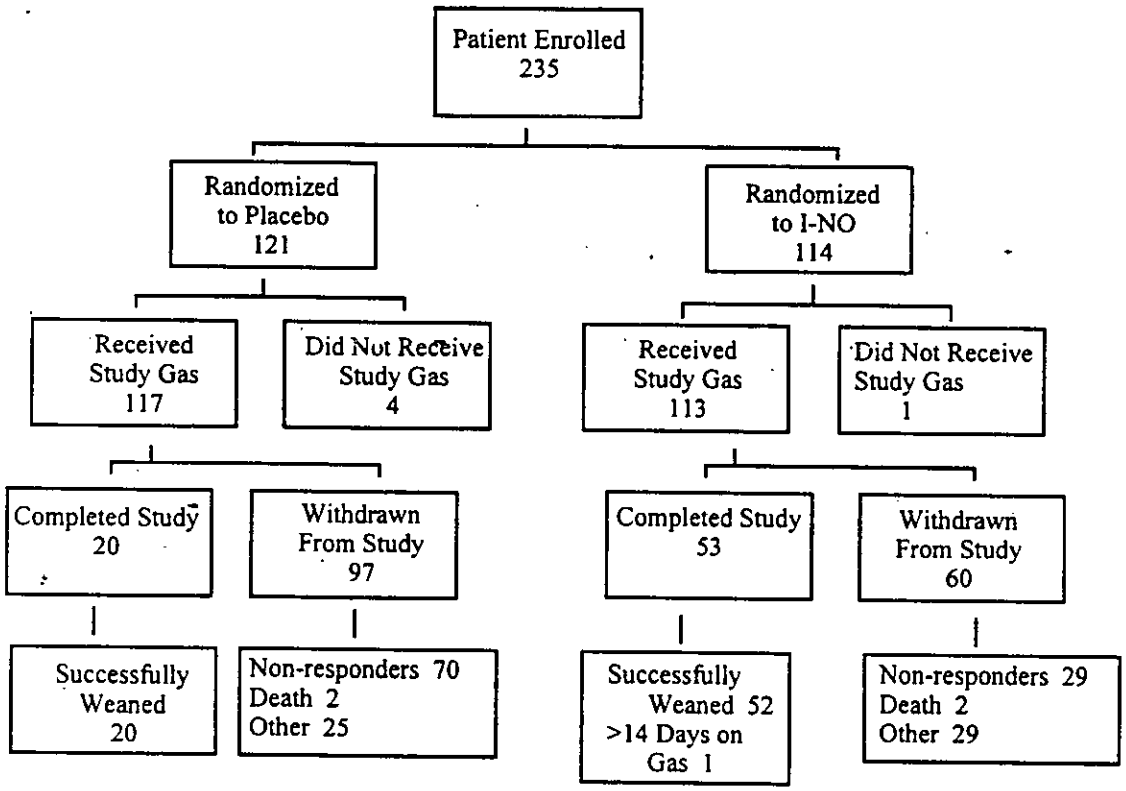
6.0.1.12.2d Primary efficacy analyses of the NINOS trial results

The subjects in each response category of the study are given in the figure below.

Figure 6.0.1.12.2d.1 Disposition of Subjects in the NINOS trial



The table below summarizes the results of the NINOS trial from the primary and secondary endpoints. This analysis was done on the Intent-to-Treat study population.

Table 6.0.1.12.2d.2 Incidence of Primary and secondary endpoints from NINOS[b].

	Control n=121	I-NO Therapy n=114	p value[a]
Met combined primary endpoint[a]	77 (63.6%)	52 (45.6%)	0.0061
Death	20 (16.5%)	16 (14%)	0.596
Met criteria for transfer to ECMO[c]	83 (69%)	67 (59%)	0.12
Received ECMO	66 (54.5%)	44 (38.5%)	0.014
Change in PaO_2 (mmHg)[d]	9.7±51.7	58.2±85.2	<0.001
Change in OI[d]	0.8±21.1	-14.1±21.0	<0.001
Change in A-a DO_2 (mmHg)[d]	-6.7±57.5	-60.0±85.1	<0.001
Among survivors:			
Length of hospital stay (days)	29.5±23	36.4±45	0.17
Duration of assisted ventilation (days)	11.1±13	12.3±14	0.47
Chronic lung disease	14 (14%)	16 (16%)	0.71
Air-leak	15 (14%)	17 (16%)	0.62
Supplemental O_2 at time of discharge	15 (15%)	14 (14%)	0.89

a. Primary endpoint: death before discharge or 120 days (whichever comes first) and/or the initiation of ECMO, analyzed by chi-square test.

b. Data from NDA, volume 2.14 and SAS primary datasets. No data is available on the secondary endpoint for neurodevelopmental outcomes, which are to be assessed after 18-24 months. Percentages are calculated from # of subjects with data.

c. See below for details of ECMO criteria met.

d. Change in this parameter measured from baseline to 30 minutes.

6.0.1.12.2d Primary efficacy analyses of the NINOS trial results (cont)

Table 6.0.1.12.2d.3 Oxygenation endpoints from NINOS trial[b,c].

	Control (n=121)	I-NO Therapy (n=114)	p value
Acute changes in oxygenation			
% of subjects with full response to study gas[a]	17/117 (14.5%)	57/113 (50%)	<0.001
% of subjects full or partial response to study gas[a]	47/117 (40%)	81/113 (72%)	<0.001
Change in PaO_2 (mmHg)[d]	9.7±51.7	58.2±85.2	<0.001
Change in OI[d]	0.8±21.1	-14.1±21.0	<0.001
Change in A-a DO_2 (mmHg)[d]	-6.7±57.5	-60.0±85.1	<0.001

a. Determination of response was made after 30 minutes on study gas:
Full response: > 20 mmHg increase in PaO_2.
Partial response: 10-20 mmHg increase in PaO_2.
No response: <10 mmHg increase in PaO_2.
b. Note: pCO_2 and pH were not collected in the NINOS trial.
c. p values calculated by chi-square or Student's t-test as appropriate.

The infants were all evaluated for a series of pre-specified criteria, meant to guide the investigator in the need for ECMO. All of the children who received ECMO met the criteria for receiving it. The majority of infants met the criteria for ECMO with an elevated OI. Not all infants who met the criteria for needing ECMO, however, received ECMO. The final decision to provide ECMO was made by the individual investigators.

Importantly, the NINOS protocol did not specify when infants were to be evaluated for ECMO. This meant that some infants were analyzed before receiving study gas, while others were assessed at unknown amounts of time after initiating study gas (personal conversation with Dr. Ehrenkranz, NINOS principle investigator). No record was kept of when the evaluation of need for ECMO was performed.

Table 6.0.1.12.2d.4 ECMO criteria met by subjects in NINOS trial.

	Control (n=121)	I-NO Therapy (n=114)	p value
Met ECMO criteria	83/121 (69%)	67/114 (59%)	0.12
ECMO criteria met			
OI >40 twice in at least 30 minutes, or OI >35 for 4 hours	63/83 (76%)	53/67 (79%)	0.64
A-aDO_2 >630 for 4 hours or >620 for 12 hours	2/83 (2%)	3/67 (5%)	
Lack of response to treatment gas[a]	11/83 (13%)	5/67 (8%)	
Other criteria	7/83 (8%)	6/67 (9%)	
Transported for ECMO	12/121 (10%)	9/114 (8%)	0.59
Received ECMO	66/121 (55%)	44/114 (39%)	0.014

a. Lack of response defined as any two of the following: 1. PaO_2 <55 for >2 hours; 2. pH <7.15 or <7.40 if alkalosis was attempted for > 2 hours; 3. mean systemic BP <40 for > 2 hours; 4. severe barotrauma.

As shown in the table below, the most common reason for an infant meeting the criteria for, but not receiving, ECMO, was 'Improved', which occurred more frequently in the infants receiving I-NO. No further details concerning this 'improvement' were recorded.

Table 6.0.1.12.2d.5 Reasons subjects met criteria for ECMO but did not receive it in NINOS trial[a].

	Control	I-NO Therapy)	p value[b]
Number meeting ECMO criteria but not receiving ECMO	17/121 (14%)	23/114 (20%)	0.065
Died prior to ECMO	2 (12%)	2 (9%)	0.46
Improved	8 (47%)	14 (61%)	
Support withdrawn	2 (12%)	1 (4%)	
Contraindications for ECMO	1 (6%)	4 (17%)	
Other reasons	4 (24%)	2 (9%)	

a. Data from NDA volume 2.14, page 028008.
b. p value calculated using unadjusted chi-square.

6.0.1.12.3 Sub-group and Post-hoc Efficacy Analyses of the NINOS Trial Results

6.0.1.12.3a Analysis according to actual study gas received

The analysis above is based on the Intent-to-Treat (ITT) population: the subjects were analyzed according to the category they were randomized to (control gas or I-NO), regardless of whether they received the gas, or whether they received the correct type of gas. In the course of the trial, however, five individuals were randomized but did not receive gas. Another 6 infants received I-NO after being randomized to control, and one infant received control gas after being randomized to receive I-NO.

a. Individuals who did not receive study gas: (data from NDA volume 2.15, page 048408):

1. Center 56, Network 120, Subject # A11. Infant was randomized to control gas despite OI<25. Study gas never started.

2. Center 15, Network 27, Subject #A19. Infant with coarctation of the aorta (an exclusion criteria) picked up on ECHO after randomization to control gas. Study gas never started.

3. Center 51, Network 2, Subject # A13. After randomization to control gas, it was discovered that infant was being ventilated improperly. When ventilator was fixed, infant improved dramatically. Study gas never started.

4. Center 57, Network 101, Subject # A01. Infant randomized to control gas, but died before study gas was administered. Study gas never started.

5. Center 58, Network 110, Subject # A01. Infant randomized to I-NO, then ECHO revealed coarctation of the aorta (exclusion criteria). Study gas never started.

b. Individuals who received the 'incorrect' gas: (data from NDA volume 2.14, page 032908).

The first group of infants were randomized to control gas, and instead received I-NO. Six of these seven subjects who incorrectly received I-NO ultimately received ECMO and two of the seven died. The specified intent-to-treat analysis above attributes these events to the control group, when in fact the infants received I-NO.

1. Center 5, Network 9, Subject # A09. Infant with meconium aspiration randomized to control gas on day 11, but received I-NO. The infant met criteria for ECMO and ECMO was started on day 15, 94 hours after starting study gas. The infant was discharged on day 34.

2. Center 12, Network 3, Subject # A02. This infant with meconium aspiration was randomized to control gas but received I-NO (up to 80 ppm). She met the criteria for ECMO and received ECMO after 6 hours on study gas. She was discharged on day 22.

3. Center 14, Network 2, Subject # A02. This infant with pneumonia and sepsis was randomized to control gas, but received I-NO on day 2. The infant met the criteria and received ECMO the same day. He died on day 21.

4. Center 51, Network 3, Subject # A19. Infant with meconium aspiration was randomized to control gas, but received I-NO. She met the criteria for and received ECMO 2 hours and 26 minutes after study gas was initiated. The infant was discharged on day 54.

5. Center 54, Network 138, Subject # A08. Infant with meconium aspiration was randomized to control gas, but received I-NO. He met the criteria for, and received ECMO 35 hours and 54 minutes after study gas was started. He was discharged on day 38.

6. Center 54, Network 153, Subject # A14. Infant with pneumonia/sepsis and pulmonary hemorrhage was randomized to control gas, but received I-NO. She had a full response to I-NO, 80 ppm. After 70 hours on study gas, her condition deteriorated and she met criteria for ECMO (by OI criterion). She then improved, so that ECMO was not attempted, but then crashed acutely, became to unstable to transport for ECMO, and died.

7. Center 55, Network 69, Subject # A13. Infant with Respiratory Distress Syndrome, was randomized to control gas, but received I-NO. The infant did not respond to I-NO at 20 or 80 ppm, met the criteria for and received ECMO, after 4 hours and 25 minutes on study gas. The infant was discharged on day 35.

One infant was randomized to receive control gas, after first receiving I-NO. Because this infant received I-NO initially, this infant is considered to have received I-NO for purposes of later statistical analysis.

8. Center 52, Network 56, Subject # A07. Infant with meconium aspiration was randomized to, and received I-NO. After a partial response to I-NO, 20 and 80 ppm, the infant met criteria for use of ECMO, and was transferred to another center for ECMO. There, another trial of study gas was made, and the infant incorrectly received control gas, rather than I-NO. The infant later received ECMO.

6.0.1.12.3a Analysis according to actual study gas received

An analysis of the data according the what gas the infants actually received is below. In this analysis, the infants who were randomized, but did not receive study gas, are excluded. The infants who received I-NO (correctly or incorrectly according to randomization) are grouped together, as are the infants who received control gas. The last individual, who received both control and I-NO gas, is attributed to the I-NO group. This analysis, grouping the subjects according to the gas actually received reduces, but does not eliminate, the statistical significance of the primary endpoint. Also note that there is still no difference in the incidence of death in the two populations.

Table 6.0.1.12.3a.1 Analysis of primary endpoints by type of study gas received in NINOS[b].

	Control	I-NO Therapy	p value
Met combined primary endpoint[a]	71/112 (63%)	56/118 (47%)	0.015
Death	17/112 (15%)	17/118 (14%)	0.869
Received ECMO	62/112 (55%)	48/118 (41%)	0.026

a. Primary endpoint: death before discharge or 120 days (whichever comes first) and/or the initiation of ECMO.
b. Data from electronic datasets, analyzed using unadjusted chi-squared test.

Finally, if the Cochran-Mantel-Hanszell adjusted chi-squared test is used, to account for variability between centers, an analysis of the primary endpoint again showed a significant difference between the control and I-NO groups (p value = 0.022).

6.0.1.12.3b Subject response by etiology, demographics or specific therapy

Extending the primary analysis, the sponsor performed a series of subgroup analyses, based on the primary endpoint.

Table 6.0.1.12.3b.1 Incidence of subjects meeting the primary endpoint (death and/or initiation of ECMO) within specific subgroups of the NINOS study population[c].

	Control	I-NO Therapy	Relative Risk	95% C.I.[a]	p value
Analysis by etiology					
PPHN (n=41)	16/21 (76%)	6/20 (30%)	0.39	0.21-0.73	0.11
Meconium Aspiration Syndrome (MAS) (n=116)	36/59 (61%)	30/57 (53%)	0.86	0.63-1.19	
Pneumonia/sepsis (n=50)	16/24 (67%)	10/26 (39%)	0.58	0.33-1.00	
RDS (n=25)	7/15 (47%)	5/10 (50%)	1.07	0.46-2.49	
Analysis by demographics					
Delivery method					
Vaginal delivery (n=119)	43/63 (68%)	25/66 (45%)	0.65	0.47-0.9	0.433
Cesarean section (n=116)	34/58 (59%)	27/58 (47%)	0.79	0.56-1.13	
Sex					
Male (n=139)	49/75 (65%)	31/64 (48%)	0.74	0.55-0.99	0.779
Female (n=96)	28/46 (61%	21/50 (42%)	0.69	0.46-1.02	
Race					
White (n=142)	41/72 (57%)	33/70 (47%)	0.83	0.6-1.14	0.245
Non-white (n=88)	35/47 (74%)	19/41 (46%)	0.62	0.44-0.88	
Analysis by initial OI					
Initial OI 25-30 (n=53)	17/28 (61%)	7/25 (28%)	0.46	0.24-0.88	0.672
Initial OI <40 (n=126)	32/64 (50%)	24/63 (39%)	0.77	0.52-1.15	
Initial OI>40 (n=108)	45/57 (79%)	28/51 (55%)	0.70	0.53-0.91	

a. C.I.: confidence interval.
b. Includes HFOV and HFJV..
c. Data from NDA volume 2.14, Table T-1.

6.0.1.12.3b Subject response by etiology, demographics or specific therapy (cont)

Table 6.0.1.12.3b.1 Incidence of subjects meeting the primary endpoint (death and/or initiation of ECMO) within specific subgroups of the NINOS study population[c] (cont).

	Control	I-NO Therapy	Relative Risk	95% C.I.[a]	p value
Analysis by Specific Therapy					
Surfactant					
Used (n=168)	47/87 (54%)	31/81 (38%)	0.72	0.55-0.95	0.94
Not used (n=67)	30/34 (88%)	21/33 (64%)	0.71	0.51-0.99	
High-frequency ventilation[b]					
Used (n=130)	44/68 (65%)	29/62 (47%)	0.72	0.53-0.99	0.94
Not used (n=105)	33/53 (62%)	23/52 (44%)	0.71	0.49-1.02	
Both surfactant and high-frequency ventilation					
Both used (n=147)	50/73 (68%)	37/74 (50%)	0.73	0.56-0.96	0.744
Both not used (n=88)	27/48 (56%)	15/40 (37.5%)	0.67	0.42-1.05	
Steroids					
Used (n=34)	10/19 (53%)	4/15 (27%)	0.51	0.21-1.23	0.433
Not used (n=201)	35/102 (34%)	67/99 (66%)	0.74	0.57-0.94	
Tolazoline					
Used (n=40)	10/17 (59%)	9/23 (39%)	0.67	0.35-1.28	0.772
Not used (n=194)	66/103 (64%)	43/91 (64%)	0.74	0.57-0.95	

a. C.I.: confidence interval.
b. Includes HFOV and HFJV.
c. Data from NDA volume 2.14, Table T-1.

6.0.1.12.3c Subject response by baseline clinical characteristics

Extending the primary analysis, these analyses on the subjects grouped by clinical characteristics, were based on the primary endpoint.

Table 6.0.1.12.3c.1 Incidence of subjects meeting the primary endpoint within specific clinical subgroups of the NINOS study population.

	Control	I-NO Therapy	Relative Risk	95% C.I.[a]	p value
Analysis by oxygenation parameters					
Initial OI 25-30 (n=53)	17/28 (61%)	7/25 (28%)	0.46	0.24-0.88	0.672
Initial OI <40 (n=126)	32/64 (50%)	24/63 (39%)	0.77	0.52-1.15	
Initial OI>40 (n=108)	45/57 (79%)	28/51 (55%)	0.70	0.53-0.91	
Analysis by baseline ECHO					
Pulmonary hypertension present	62/95 (65%)	38/81 (47%)	0.72	0.55-0.99	0.94
Pulmonary hypertension absent	11/22 (50%)	11/28 (39%)	0.79	0.55-0.95	

a. C.I.: confidence interval.

6.0.1.12.3d Initial Response to 'low flow gas'

It has been proposed that the acute change in oxygenation parameters can be used as a surrogate for long-term clinical outcome following I-NO exposure. The table below summarizes the subjects in the two groups who met the primary endpoint (Death and/or ECMO), and for ECMO alone, grouped by the acute response to study gas. Note that for individuals who did not respond to study gas, there was no reduction in the rate of death and/or the initiation of ECMO.

Table 6.0.1.12.3d.1 Analysis of subjects who met primary endpoint (Death and/or ECMO), or received ECMO grouped by initial response to study gas[a].

	Placebo Group		I-NO Group	
	Primary Endpoint	ECMO	Primary Endpoint	ECMO
Full Response[a]	9/17 (53%)	8/17 (47%)	14/57 (24%)	1/57 (2%)
Partial Response	4/13 (31%)	3/13 (23%)	9/17 (47%))	8/17 (47%)
No Response	63/87 (72%)	55/87 (63%)	27/38 (71%)	22/38 (58%)

a. Determination of response was made after 30 minutes on study gas:
Full response: > 20 mmHg increase in PaO_2.
Partial response: 10-20 mmHg increase in PaO_2.
No response: <10 mmHg increase in PaO_2.

6.0.1.12.3e Incidence of death in subjects who received ECMO

To see if I-NO had any effect on survival of infants that required ECMO, the incidence of death in two groups was compared: the control and I-NO groups who received ECMO.

Table 6.0.1.12.3e.1 Incidence of death among recipients of ECMO from the NINOS trial.

	Control	I-NO Therapy	p value[a]
Incidence of death	9/66 (14%)	8/36 (22%)	0.27

a. p value using unadjusted chi-square test on the ITT population.

6.0.1.12.3f Analysis of the subjects who met criteria for, but did not receive ECMO

A pivotal part of the NINOS results rests with the group of individuals who met criteria for, but did not receive, ECMO. In the study, there were 40 subjects who were found to meet the criteria for, but did not receive, ECMO. Of these, 16 received control gas and 24 received I-NO. As detailed in Table 6.0.1.12.2d.5 above, the most common reason for not receiving ECMO was clinical improvement, as judged by the individual investigators. No other information, including oxygenation parameter or vital signs, are available regarding this decision. It is of interest, however, to see if the two groups of individuals were similar prior to receiving study gas. The table below shows the baseline clinical parameters for the two groups, collected prior to initiation of study gas. The higher OI value in the control group derived from 2 subjects with exceptionally high OI values (157 and 139) at baseline. Both subjects died, one suddenly before ECMO could be provided, and one after the parents denied permission for ECMO. Absent these two individuals, the baseline characteristics are similar in the two groups.

Table 6.0.1.12.3f.1 Baseline clinical characteristics of subjects who met criteria for, but did not receive, ECMO[a].

Treatment Group	PaO_2	OI	MAP[b]	A-a DO_2
Control Gas (n=16)	35.6±12.7	60.3±37	17.9±4.8	624.5±39
I-NO (n=24)	39.1±11.4	47.0±16.1	17.1±3.96	633.8±18

a. Data from electronic datasets and NDA volumes 2.14 & 2.15. Blood pressure and heart rate data were not collected.
b. Mean Airway Pressure in cm H_2O.

6.0.1.13 Safety comparisons

6.0.1.13.1 Comparison of defined safety parameters up to 28 days

Safety Parameters:

1. Methemoglobin levels.
2. Inhaled NO_2 concentrations.
3. Incidence of air leak syndrome (pneumothorax, pneumopericardium, pneumoperitoneum, pneumomediastinum, interstitial emphysema).
4. Incidence of chronic lung disease (defined as O_2 >21% required at 28 days of age with abnormal chest x-ray).
5. Incidence of intracranial abnormalities and risk factors for abnormal neurological sequelae (seizures, intraventricular hemorrhage and brain infarct).
6. Incidence of pulmonary or gastrointestinal hemorrhage.

The first table shows the incidence of certain clinically relevant condition of the NINOS subjects noted prior to beginning study gas. The table is taken from earlier in this review. A numerically larger number of subjects in the control group had brain infarcts and/or intraventricular hemorrhage at baseline.

Table 6.0.1.13.1.1 Other conditions noted prior beginning study gas in NINOS.

Characteristic	Placebo Group (n=121)	I-NO Group (n=114)	p value[a]
Air leak syndrome	25 (21%)	20 (18%)	0.54
Pulmonary hemorrhage	21 (17%)	18 (16%)	0.75
Prolonged oozing	2 (2%)	6 (5%)	0.13
GI bleeding	1 (1%)	5 (4%)	0.08
Seizures starting after randomization	20/121 (17%)	13/114 (11%)	0.26
Seizures requiring therapy	7 (6%)	12 (11%)	0.18
Brain Infarct	2/88 (2%)	0/82 (0%)	0.17
Interventricular hemorrhage (IVH)[a]	9/87 (10%)	3/82 (4%)	0.10
IVH Grade I	8/9 (89%)	3/3 (100%)	0.10
IVH Grade II	1/9 (11%)	0/3 (0%)	0.55
Periventricular leukomalacia	2/88 (2%)	0/82 (0%)	0.17

a. An equal number of subjects in both treatment groups were evaluated with cranial ultrasound (73%). Thus, the total number of evaluations in the control group was 88/121 (73%) and in the I-NO was 82/114 (73%).

6.0.1.13.1 Comparison of defined safety parameters up to 28 days (cont)

The next table shows the incidence of the same safety parameters that occurred during the course of the trial. Not all subjects have data for a given parameter. Note the numerical and percentage increase in Air Leak Syndrome (ALS) during I-NO administration, relative to control gas. Otherwise, there is no suggestion that the two groups differ with respect to the occurrence any of the major safety endpoints prospectively identified by the investigators as 'of interest'.

Table 6.0.1.13.1.2 Comparison of specific safety parameters during the NINOS trial[a].

Characteristic	Placebo Group (n=121)	I-NO Group (n=114)	p value[a]
Air leak syndrome during study gas administration[b]	7/121 (6%)	12/110 (11%)	0.23
Airleak syndrome during and after study gas[d]	19/121 (16%)	21/110 (19%)	
Pulmonary hemorrhage	4/110 (4%)	2/107 (2%)	0.43
Chronic lung disease[c]	15/121 (12%)	16/114 (14%)	0.85
Discharge home on O_2	15/100 (15%)	14/98 (14%)	0.89
Prolonged oozing	8/109 (7%)	5/107 (5%)	0.41
GI bleeding	1/109 (1%)	1/107 (1%)	0.99
Seizures requiring therapy	20/122 (17%)	13/114 (11%)	0.26
Brain Infarct	4/82 (5%)	7/77 (9%)	0.30
Interventricular hemorrhage (IVH)[a]	21/108 (19%)	16/111 (14%)	0.48
IVH Grade I	10/21 (62%)	9/16 (56%)	0.74
IVH Grade II	3/21 (14%)	0/16 (0%)	N/A
IVH Grade III-IV	8/21 (38%)	7/16 (44%)	0.75
Periventricular leukomalacia	3/82 (4%)	4/77 (5%)	0.73

a. Unless otherwise noted, the data shown is for adverse events which occurred after randomization. P-value calculated using chi-square test.

b. Incidence of pulmonary leak occurring up to 24 hours after discontinuation of study gas. Does not include 21 subjects (12 control, 9 I-NO) who developed air leak >24 hours after discontinuation of study gas.

c. Chronic lung disease (CLD) defined as O_2 >21% required at 28 days of age with abnormal chest x-ray).

d. Incidence of pulmonary leak occurring up to time of discharge, starting after initiation of study gas.

6.0.1.13.2 Comments on specific safety parameters

The next section will summarize the results for the safety endpoints followed in the trial. p values are calculated using unpaired Student's t-test or chi-square test as appropriate, unless otherwise stated.

6.0.1.13.2a NO_2 concentrations

Only one individual had a NO_2 level >7.0 % during the trial (subject #A08 from center 55). The level was 9.1, and the subject underwent a successful wean of study gas.

Table 6.0.1.13.2a.1 Peak NO_2 levels in ppm from the NINOS trial.

Changes in safety endpoints	Control	Combined I-NO	p value
Peak NO_2 level during first 12 hours of study gas	0.1±0.3	0.6±0.9	<0.001
Peak NO_2 level at any time	0.1±0.3	0.8±1.2	<0.001
Peak NO_2 level at any time			
0.0 - 1.0	98/101 (97%)	85/110 (77%)	<0.001
1.1 - 3.0	3/101 (3%)	21/110 (19%)	
3.1 - 5.0	0/101 (0%)	2/110 (2%)	
5.1 - 7.0	0/101 (0%)	1/110 (1%)	
7.1 to 10	0/101 (0%)	1/110 (1%)	
Peak NO_2 level at any time, excluding 8 subjects who received wrong study gas	0.0±0.3	0.8±1.2	<0.001

6.0.1.13.2b Methemoglobin concentrations

Table 6.0.1.13.2b.1 Peak Methemoglobin levels from the NINOS trial.

Changes in safety endpoints	Control	Combined I-NO	p value
Peak methemoglobin level during first 12 hours of study gas	1.0±0.6	2.0±1.5	<0.001
Peak methemoglobin level at any time	1.2±0.8%	2.4±1.8%	<0.001
Peak methemoglobin level at any time			
0.0 - 1.0%	52/112 (46%)	15/110 (14%)	<0.001
1.1 - 2.0	49/112 (44%)	49/110 (45%)	
2.1 - 30	6/112 (5%)	23/110 (21%)	
3.1 - 5.0	4/112 (4%)	12/110 (11%)	
5.1 to 10	1/112 (1%)	11/110 (10%)	
Peak methemoglobin level at any time, excluding 8 subjects who received wrong study gas	1.2±0.8%	2.4±1.8%	<0.001

A total of 11 subjects (4 controls, 7 I-NO) had their study gas decreased because their methemoglobin levels were >5%. All continued on study gas at lower flow rate. No subject was discontinued because of NO_2 >7 ppm or methemoglobin >10%.

6.0.1.13.2c Subject deaths

No case report forms or narratives for the 36 deaths in the NINOS trial are available. The cause of death for the infants was recorded for each infant by the investigator, and is shown below.

Table 6.0.1.13.2c.1 Cause of death for the 36 infants in the NINOS trial[a].

Cause	Placebo Group	I-NO Group
All causes	20/121 (16%)	16/114 (14%)
Respiratory distress syndrome	1/20 (5%)	2/16 (13%)
Bronchopulmonary dysplasia	1/20 (5%)	0/16 (0%)
Malalignment of the pulmonary veins	2/20 (10%)	1/16 (6%)
Suspected sepsis/infection	4/20 (20%)	1/16 (6%)
Proven sepsis/infection	2/20 (10%)	4/16 (25%)
Severe intracranial hemorrhage	2/20 (10%)	0/16 (0%)
Withdrawal of support	3/20 (15%)	4/16 (25%)
Other causes	5/20 (25%)	4/16 (25%)

a. Data from NDA volume 2.14, page 031008.