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

No individual has NO_2 levels >5.0 ppm. Subject 03-59003 had levels >3.0 on several occasions during I-NO therapy, with a peak of 3.3 after 11.6 hours. The infant was discharged with both reactive airways disease and broncho-pulmonary dysplasia.

2. Incidence of intraventricular hemorrhage and seizures

Inverventricular hemorrhage was detected in 12% of the infants (1/8 with available data).

Seizures occurred in 3/14 (21%) of the infants.

The relationship of these adverse events to the administration of I-NO is difficult to establish with these small numbers. The overall NDA database includes these subjects, and will address this issue in sections 8.1 and 8.2 below.

3. Laboratory evaluations

These will be included in the analysis of lab values in section 8.1.

4. Incidence and relationship of all adverse events and specific adverse events to I-NO

These will be included in the analysis of lab values in section 8.1.

Of the other adverse events noted by the investigators, the following adverse events which occurred will be noted here, and included in section 8.3 as well.

1. Asthma

Asthma was identified in the INO-03 trial. Subject 03-59001 received I-NO 5 ppm for 73 hours, and developed asthma 1 week after starting I-NO. The infant did not receive ECMO, HFOV, or HFJV, but required supplemental O_2 at the time of discharge as well as bronchodilator therapy. Long-term follow-up is not available.

2. Air leak syndrome/pneumothoraces

28% (4/14) of the subjects in the INO-03 trial had experienced a pneumothorax at the end of 28 days.

Table 6.0.4.13.1.1 Listing of pneumothoraces in the INO-03 trial[a].

Subject group	Duration of I-NO therapy	Notes
I-NO 5 ppm		
03-57003	120 hours	No ECMO, HFOV/HFJV
03-59004	32 hours	No ECMO, HFOV/HFJV
I-NO 20 ppm		
03-58001	16 hours	ECMO
03-59002	168 hours	No ECMO, HFOV/HFJV Required O_2 at 28 days, BPD

a. Data from NDA volumes 2.30 and 2.31.

3. PPHN Sequelae

The percentage of each group which had one of the PPHN sequelae is listed below.

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

Details of these adverse events will be included in section 8.1 and 8.2.

Table 6.0.4.13.1.2 Comparison of the rates of specific safety parameters from INO-03.[a] Note that not all subjects have data for a given parameter.

Changes in safety endpoints	I-NO 5 ppm	I-NO 20 ppm	I-N0 80 ppm	Combined I-NO
Incidence of methemoglobinemia >5%	0/4 (0%)	0/8 (0%)	1/2 (50%)	1/14 (7%)
Incidence of elevated NO_2 level (>5 ppm)[a]	0/4 (0%)	0/8 (0%)	0/2 (0%)	0/14 (0%)
Incidence of seizures	0/4 (0%)	3/7 (43%)	0/2 (0%0	3/14 (21%)
Incidence of air leak syndrome[c]	2/4 (50%)	2/8 (25%)	0/2 (0%)	4/14 (28%)
Incidence of bronchopulmonary dysplasia[d]	0/4 (0%)	1/7 (14%)	1/2 (50%)	2/14 (14%)
Subjects requiring O_2 at 28 days	0/4 (0%)	1/7 (14%)	1/2 (50%)	2/14 (14%)
Subjects with reactive airways disease at 28 days	0/4 (0%)	1/7 (14%)	1/2 (50%)	2/14 (14%)
Incidence of sensorineural hearing loss[e]	0/4 (0%)	2/8 (25%)	1/2 (50%	3/14 (21%)
Intracranial abnormalities detected by ultrasound, CT or MRI scan				
Abnormality on cranial ultrasound[b]	0/3 (0%)	1/4 (25%)	0/1 (0%)	1/8(12%)
Interventricular hemorrhage	0/3 (0%)	1/4 (25%)	0/1 (0%)	1/8(12%)
Intracranial infarct detected by CAT scan or MRI	0/3 (0%)	1/4 (25%)	0/1 (0%)	1/8(12%)

a. Occupational health guidelines have set an eight hour maximum exposure limit at 5 ppm for nitrogen dioxide (NO_2)(76).

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. Air leak syndrome includes the occurrence of any one of the following: interstitial emphysema; pneumomediastinum; pneumopericardium; and pneumothorax. In this trial, the only abnormality noted was pneumothorax.

d. Bronchopulmonary dysplasia defined as: use of supplemental O_2 at 28 days of life in the presence of an abnormal CXR, or the use of bronchodilators suggesting severe reactive airway disease on discharge.

e. Sensorineural hearing loss was detected using brain stem auditory evoked responses (BAER).

4. Subject deaths: no subject deaths occurred in the INO-03 trial

6.0.4.14 INO-03 Efficacy summary

The primary intent of the INO-03 study was to collect further safety data. This, coupled with the absence of a control group, and the small number of subjects entered into the trial, limit the information regarding efficacy in this trial.

Fifty-seven % of the subjects in the INO-03 trial met one of the four primary PPHN endpoints. This compares with 56% of the control subjects, and 50% of the I-NO group in the INO-01/ -02 trial.

None of the infants in the INO-03 trial died, compared with 2% of the control group in the INO-01/ -02 trial, and 8% of the I-NO group in the INO-01/ -02 trial.

Twenty-one % of the subjects in the INO-03 trial received ECMO, compared with 39% of the control subjects in the INO-01/ -02 trial, and 29% of the I-NO group in the INO-01/ -02 trial.

Overall, the infants in the INO-03 trial had similar incidence rates for the primary endpoints to those seen in the INO-01/ -02 trial.

6.0.4.15 INO-03 Safety summary

No deaths, and no unanticipated adverse events were identified in this small study.

The safety data from this study (summarized in Table 6.0.4.13.1.2) will be incorporated into the overall safety database in sections 8.1 and 8.2 below.

6.0.4.16 INO-03 Reviewer's Summary

1. 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.

2. No adverse events were seen which did not also occur in the other, larger, trials. The small numbers of subjects preclude the use of statistics to analyze the safety data, but the safety information agreed in large part with the other trials. The rates of occurrence for specific adverse events were tabulated, and will be incorporated into the larger, overall Safety Review in sections 8.1 and 8.2.

7.0 Integrated Review of Efficacy

There are three aspects of the determination of efficacy for I-NO: meeting pre-specified primary endpoints; demonstrating physiological effect; and demonstrating clinical benefit. The latter two aspects of efficacy were included in the secondary and exploratory endpoints of the three trials. A summary of the primary and secondary endpoints of the NINOS, INOSG and INO-01/ -02 trials is below.

7.0.1 Primary and secondary efficacy endpoints from the NINOS, INOSG, and INO-01/ -02 trials

Primary efficacy endpoints

I. NINOS primary endpoint

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

II. INOSG primary endpoint

1. Number of acute oxygenation 'successes' following 20 minutes of treatment gas.

III. INO-01/ -02 primary endpoint

1. The occurrence of one or more of the PPHN major sequelae prior to discharge:
    a.. Death.
    b. Initiation of ECMO.
    c. Evidence of abnormal neurological sequelae.
    d. Bronchopulmonary dysplasia.

Secondary efficacy endpoints

I. NINOS 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 $Aa\text{-}DO_2$ levels before and 30 minutes after initial administration of the study gas.
4. Neurodevelopmental outcomes assessed at 18-24 months corrected age (data not yet submitted).
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.

II. INOSG Secondary and post-hoc analyses

1. The number of subject deaths within 120 days and/or receipt of ECMO
2. Percentage of subjects receiving oxygen therapy at 28 days.
3. Percentage of subjects surviving.

IIIa. I-NO-01/ -02 secondary endpoints

1. Physiologic response to I-NO, measured by change in OI and time-weighted OI.
2. Number of days requiring supplemental oxygen.
3. Number of days requiring mechanical ventilation.
4. Number of days in hospital (defined as to end of medically indicated hospitalization, not related to social issues).

IIIb. I-NO-01/ -02 long-term follow-up endpoints (measured at 1 year follow-up examination)

1. Incidence of hearing abnormalities.
2. Incidence of developmental delay.

IIIc. I-NO-01/ -02 exploratory variables

1. Postductal PaO_2.
2. Preductal O_2 saturation.
3. Postductal O_2 saturation.
4. Mean Arterial Pressure.
5. Positive Inspiratory Pressure.
6. Positive End-Expiratory Pressure (PEEP).
7. Arterial-alveolar O_2 ratio.
8. Arterial-alveolar O_2 gradient.

7.0.1 Success of trials in meeting pre-specified primary endpoints

Two of the three trials submitted in support of efficacy met their pre-specified primary endpoint: the NINOS and INOSG trials. Of these, only the NINOS trial endpoint was previously held to be of sufficient clinical benefit to support approval of I-NO. The INOSG endpoint, acute improvement in oxygenation, was felt by the Cardiovascular and Renal Drugs Advisory Committee to be an inadequate endpoint to demonstrate clinical efficacy (see section 2.3).

The table below summarizes the rates of the pre-specified, primary endpoints from the three efficacy trials in NDA 20845[d].

Table 7.0.1.1 Primary endpoints from the NINOS, INOSG, and INO-01/ -02 trials.

Study Endpoint	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value
NINOS: death before 120 days and/or initiation of ECMO	70/111 (63%)				57/119 (47.9%)	0.021[a]
INOSG: acute oxygenation success	2/28 (7%)			16/30 (53%)		0.0002[b]
INO-01/ -02: PPHN major sequelae[c]	23/41 (56%)	18/36 (50%)	21/35 (60%)	13/33 (39%)	52/104 (50%)	0.34[c]

a. p value calculated from the subjects who actually received study gas, grouped according to the study gas actually received, using unadjusted chi-square.

b. p value calculated using unadjusted chi-square.

c. PPHN major sequelae: death; initiation of ECMO; bronchopulmonary dysplasia; neurologic abnormalities. p value calculated using unadjusted chi-square.

d. Data from individual study reports, sections 6.0.1, 6.0.2, and 6.0.3.

7.0.2 Analysis of the NINOS primary endpoint in the INOSG and INO-01/ -02 trials

Another way of analyzing the data is to ask whether the significant effect of I-NO as regards the NINOS primary endpoint was seen in any of the other trials. The table below summarizes that information.

Table 7.0.2.1 Post-hoc analysis of the NINOS primary endpoint (death before 120 days and/or initiation of ECMO) from the NINOS, INOSG and INO-01/ -02 databases[b].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value
NINOS[c]	77/121 (63.6%)				52/114 (45.6%)	0.006[a]
NINOS[d]	71/112 (63%)				56/118 (47.4%)	0.022[c]
INOSG	21/28 (75%)			13/30 (43%)		0.0182[a]
INO-01/ -02	16/41 (39%)	11/40 (28%)	14/36 (39%)	8/37 (22%)	33/113 (29%)	0.25[c]

a. p value calculated using unadjusted chi-square.

b. Data from individual study reports, sections 6.0.1, 6.0.2 an 6.0.3.

c. Analysis based on intent to treat (ITT) population.

d. Analysis based on 'gas received' population. p value calculated using Cochran-Mantel-Haenszel adjusted chi-square test.

a. p value calculated using one-way ANOVA.

As can be seen from the table above, while there was a significant difference in the NINOS and INOSG trials in this endpoint, no significant difference in the INO-01/ -02 trial was seen. There was a reduction in the % of subjects who met the primary endpoint in two of the three I-NO groups in the INO-01/ -02 trial, however. The table below expresses the reductions in the rate of the primary endpoint in the three trials, using the ITT population in the NINOS.

Table 7.0.2.2 Percent of subjects who met the NINOS primary endpoint (death before 120 days and/or initiation of ECMO) in the NINOS, INOSG, and INO-01/ -02 trials[a].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled
NINOS	63.6%				45.6% (-28%)
INOSG	75%			43% (-43%)	
INO-01/ -02	39%	28% (-28%)	39% (0%)	22% (-44%)	29% (-26%)

a. Percent reduction calculated as control minus I-NO/control X100.

The initiation of ECMO can also be compared across the three trials, in a search for demonstration of efficacy. The first table below shows the absolute rates, while the following table shows the % reductions from the control rate for each of the trials. Note the lower rate of use of ECMO in the INO-01/ -02 trial in the control group (34%), when compared with either NINOS (55%) or INOSG (71%).

7.0.2 Analysis of the NINOS primary endpoint in the INOSG and INO-01/ -02 trials (cont)

Table 7.0.2.3 Rate of the initiation of ECMO in the NINOS, INOSG, and INO-01/ -02 trials[b].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value[a]
NINOS[a]	66/121 (54.5)				44/114 (38.5%)	0.014
NINOS[c]	62/112 (55%)		0.067[c]		48/118 (41%)	0.067[c]
INOSG	20/28 (71%)			12/30 (40%)		0.0198
INO-01/ -02	14/41 (34%)	10/41 (24%)	9/36 (25%)	6/37 (16%)	25/114 (22%)	0.34

a. Based on ITT population, p value calculated using unadjusted chi-square.
b. Data from individual study reports, sections 6.0.1, 6.0.2 and 6.0.3.
c. Based on 'gas received' population. p value calculated using Cochran-Mantel-Haenszel adjusted chi-square test.

Table 7.0.2.4 Percent reduction in the rate of the initiation of ECMO in the NINOS, INOSG, and INO-01/ -02 trials[a].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled
NINOS	55%				41% (-25%)
INOSG	71%			40% (-44%)	
INO-01/ -02	34%	24% (-29%)	25% (26%)	16% (-53%)	22% (-35%)

a. Percent reduction calculated as control minus I-NO/control X100.

The other component of the NINOS primary endpoint, the mortality rate, is discussed in section 8.1.1. There was, however, no detected difference between the control and I-NO groups overall with regards to the mortality rate. The two overall rates calculated below yield a relative risk of 1.098 with 95% confidence interval from 0.78 to 1.54 using the method of Katz and Fisher's Exact Chi-square test.

Table 7.0.2.5 (from table 8.1.1.1) Incidence of death in the NINOS, INOSG, INO-01/ -02 and INO-03 studies[b].

Study	Control group	I-NO group	p value
NINOS (0-120 days)[d]	20/121 (16.5%)	16/114 (14%)	0.596
NINOS (0-120 days)[c]	17/112 (15.1%)	17/118 (14.4%)	0.87
INOSG (0-445 days)	3/28 (10.7%)	2/30 (6.7%)	0.70
INO-01/ -02 (0-28 day)	1/41 (2.4%)	9/113 (8%)	0.29
INO-01/ -02 (0-1 yr)	2/41 (4.9%)	10/113 (8.8%)	0.42
INO-03 (0-28 days)	No control group	0/14 (0%)	N/A
Total[a,e]	24/190 (12.6%)	27/271 (9.9%)	0.43
Total[a,f]	22/181 (12.1%)	29/275 (10.5%)	0.28

a. The comparability of the incidence rates between the trials is limited by the varying length of follow-up for each trial.
b. Data from individual study reports and electronic datasets.
c. Grouped from the NINOS subjects who actually received study gas, according to the gas actually received, using unadjusted chi-square.
d. Based on NINOS ITT population, p value calculated using unadjusted chi-square.
e. This overall incidence figure includes the ITT NINOS population, the INOSG trial, the INO-01/ -02 0-28 day population, and the INO-03 trial population.
f. This overall incidence figure includes all known deaths out to one year, using the 'gas received' population in the NINOS trial.

It is reasonable to conclude from this data that I-NO administration was associated with a significant reduction in the number of infants who were started on ECMO in the NINOS trial. The other two trials supported this effect of I-NO. In these trials, the percent reduction in the rate of both the primary endpoint and in the initiation of ECMO were reduced by amounts which were similar to those seen in the NINOS trial. The INOSG data suffers from problems with blinding and incomplete data. The INO-01/ -02 trial data did not show a significant effect of I-NO on the use of ECMO, due in part to the small numbers of subjects in each group. The subjects in the INO-01/ -02 trial were also less critically ill at time of entry, and the control infants received ECMO at a lower rate than in the INOSG or NINOS trials (see Table 6.0.3.12.1.3). This lower 'event rate' meant that a larger number of subjects would be needed to detect a significant difference between the two groups.

There was no effect of I-NO on the mortality rate detected, and this component contributed little to the overall significance for the NINOS primary endpoint.

7.0.3 Success of trials in meeting secondary efficacy endpoints: demonstrating a physiological effect of I-NO

1. Acute changes in oxygenation

The acute effect of I-NO on oxygenation, measured in all three efficacy trials, are summarized in the table below. Shown are the average changes (±standard deviation) from baseline to the first measurement after starting study gas (placebo or I-NO).

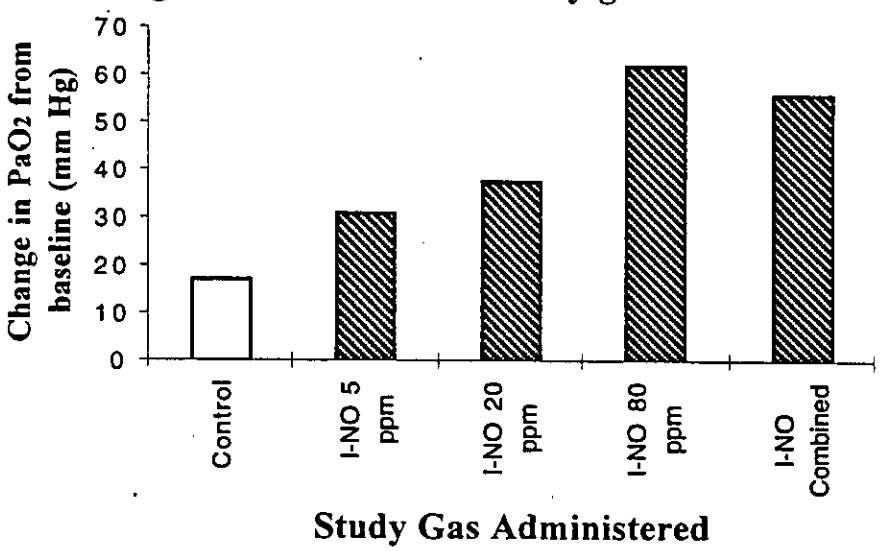
Table 7.0.3.1 Results: comparison of acute changes in oxygenation from the NINOS, INOSG and INO-01/ -02 trials[d].

'Exploratory' Variables	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Changes in clinical markers of oxygenation					
NINOS Trial[a]					
Change in PaO_2 (mmHg)[d]	9.7±51.7				58.2±85.2
Change in OI[d]	0.8±21.1				-14.1±21.0
Change in A-a DO_2 (mmHg)[d]	-6.7±57.5				-60.0±85.1
INOSG Trial[b]					
Change in PaO_2 (mmHg)	-1.9±9.6			47.4±68	
Change in OI	-2.0±15			-16±11.5	
INO-01/ -02 trial[c]					
Change in mean PaO_2	18.0±53	32.3±56	38.6±69	64.4±84	44.6±71
Change in mean OI	-1.3±7.7	-4.7±4.6	-4.3±9.6	-7.4±9.0	-5.5±8.7
Preductal O_2 saturation	0.35±4.1	0.77±3.28	0.14±3.0	0.26±3.4	0.4±3.2
Postductal O_2 saturation	0.27±4.5	1.85±3.86	1.49±4.72	1.43±3.88	1.60±4.1
Arterial-alveolar O_2 ratio	0.03±0.08	0.05±0.09	0.06±0.11	0.10±0.13	0.17±0.11
Arterial-alveolar O_2 gradient (A-aDO_2)	-19.5±56	-31.6±56	-39.6±68	-63.2±81	-44.3±70

a. Shown is the mean± s.d. of the change from baseline for each parameter, measured after 30 minutes.
b. Acute effects measured at baseline and after 20 minutes.
c. Acute effects measured at baseline and after 30 minutes.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.

The INO-01/ -02 trial also suggests that this effect of I-NO to improve oxygenation is dose-dependent in the range from 5 to 80 ppm. The figure below, based on table 6.0.3.12.3b.1, shows the change in PaO_2 following study gas administration in the INO-01/ -02 study. The change from baseline was statistically significant using one-way ANOVA (p value = 0.029).

Figure 7.0.3.2 Effect of study gas on PaO_2



7.0.3 Success of trials in meeting secondary efficacy endpoints: demonstrating physiological effect of I-NO (cont)

The long-term effects of I-NO on oxygenation were examined in the INO-01/ -02 trial, using the time-weighted OI (TWOI), a measure of the average OI/hour of study gas administration.

Table 7.0.3.3 Effects of NO on oxygenation and hemodynamics beyond 30 minutes[a] in the INO-01/ -02 trial.

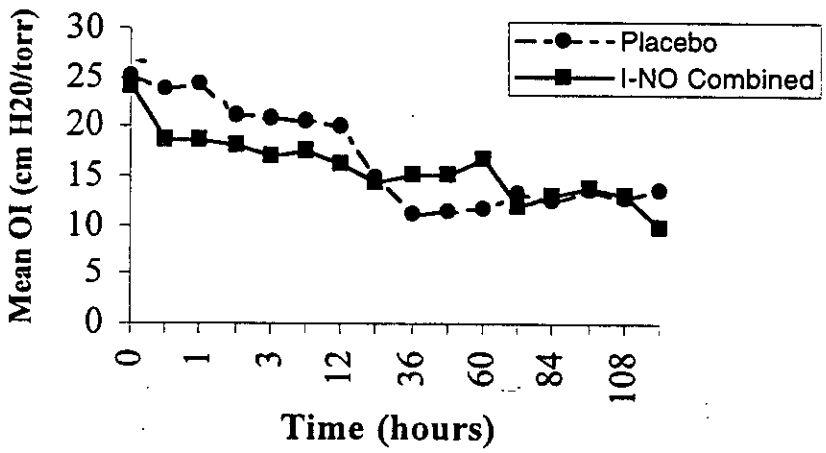
Endpoint	*Control*	*I-NO 5 ppm*	*I-NO 20 ppm*	*I-NO 80 ppm*	*Combined I-NO*	*p value*
Change in TWOI	-1.60±8.0 (-22 to 15)	-4.67±7.7 (-25 to 14)	-4.78±10.1 (-36 to 14)	-5.59±7.4 (-24 to 8)	-5.0±8.4 -36 to 14	see note[b]

a. Data taken from baseline to beyond 30 minutes to the time of withdrawal from I-NO, from NDA volume 2.17, Table 23.

b. Repeated t test comparisons with control group were significant (<0.05) for the 5 and 80 ppm group, as well as the pooled I-NO group(shaded boxes above).

The majority of this effect was manifest in the first few hours of study gas administration, where there was a clear separation of control and I-NO groups with regards to their average OI.

Figure 7.0.3.4 Time vs. OI in the INO-01/02 Trial



2. Effect of I-NO on pCO_2

The INOSG trial reported a significant, acute effect of I-NO on pH when comparing the initial baseline value to the value at the end of 20 minutes. No significant difference between the second baseline and the 20 minute value was detected. The second baseline value was taken after reducing the FiO_2 to 90%, just prior to starting the study gas. Thus, the comparison between the second baseline and the 20 minute value is the best comparison to use to determine the acute effect of I-NO, independent of any effect of reduced FiO_2. No effect of I-NO on pCO_2 was seen in the larger INO-01/ -02 trial, although there was an numerical decrease in pCO_2 in that trial in all dose-groups of I-NO. As shown in Figure 6.0.3.14.9, the average pCO_2 remained stable in both I-NO and control groups during chronic administration of study gas.

Table 7.0.3.4 Results: comparison of acute changes in pCO_2 from the INOSG, and INO-01/ -02 trials (no data submitted from the NINOS trial)[a,d].

Changes in pCO_2	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
INOSG Trial					
Change in pCO_2 from first baseline[b]	-0.9±4.8			-4.1±7.6	
Change in pCO_2 from second baseline[b]	+0.6±10			-3.2±10	
INO-01/ -02 trial[e]					
Mean change in $_PCO_2$	-0.75±5.5	-1.24±6.0	-1.31±3.6	-1.17±4.9	-1.24±4.9

a. Shown is the mean± s.d. of the change from baseline for each parameter.

b. Acute effects measured at first baseline and after 20 minutes of study gas.

c. Acute effects measured at second baseline and after 20 minutes of study gas.

d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student's t-test are shaded.

e. Acute effects measured at second baseline and after 30 minutes of study gas.

7.0.3 Success of trials in meeting secondary efficacy endpoints: demonstrating physiological effect of I-NO (cont)

3. Effect of I-NO on pH

No significant effect of I-NO on pH was detected in either the INOSG or the INO-01/ -02 trials. As shown in Figure 6.0.3.14.10, the average pH remained stable in both I-NO and control groups during chronic administration of study gas.

Table 7.0.3.5 Acute effect of I-NO on pH in the INOSG and INO-01/ -02 trials[a].

Changes in pH	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
INOSG Trial[b]					
Change in pH	0.02±0.07			0.05±0.09	
INO-01/ -02 trial[c]					
Mean pH	0.02±0.06	0.02±0.06	0.02±0.04	0.01±0.05	0.02±0.05

a. Shown is the mean± s.d. of the change from baseline for each parameter.
b. Acute effects measured at baseline and after 20 minutes.
c. Acute effects measured at baseline and after 30 minutes.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.

4. The effect of I-NO on hemodynamics and pulmonary pressures

The effects of I-NO on hemodynamics and pulmonary pressures was measured in the INOSG and INO-01/ -02 trials, which are summarized below. The INOSG trial reported an acute, significant decrease in mean systemic blood pressure only between the first baseline value and the 20 minute value. There was no significant difference between the second baseline value and the 20 minute value in the INOSG trial. The larger INO-01/ -0_2 trial found a small, non-significant decrease in mean arterial pressure in the I-NO group, and no long-term effect of I-NO on blood pressure (see figure 6.0.3.14.12).

There was no acute effect of I-NO on mean airway pressures detected in the INO-01/ -02 trial, as seen in the table below. There was also not a long-term trend towards a more rapid decrease in mean airway pressures in the I-NO group (see figure 6.0.3.14.11). Additionally, no acute or chronic effect of I-NO on the amount of PEEP required by the infants, or the average positive inspiratory pressure (PIP) was detected.

Table 7.0.3.6 Acute effect of I-NO on pulmonary and systemic hemodynamics in the INOSG and INO-01/ -02 trials.

Changes in pH	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled
INOSG Trial[b]					
Change in mean systemic blood pressure (mmHg) from first baseline[e]	+4.1±9.0			-4.6±13.2	
Change in mean systemic blood pressure (mmHg) from second baseline[f]	+1.5±10			-1.0±10	
Change in heart rate (beats per minute)	+2.0±15			+1.0±10.2	
INO-01/ -02 trial[c]					
Mean Arterial Pressure (mmHg)	-0.73±10.9	-2.39±9.69	-3.22±9.4	+0.69±10.0	-1.7±9.8
Mean Systolic Pressure	-1.32±13	-3.29±12	-4.64±14	+1.86±12	-2.0±13
Mean Diastolic Pressure	-0.68±9	-1.39±8	-2.47±7.3	-0.08±8	-1.31±8
Mean Peak Inspiratory Pressure (PIP)	+0.27±1.1[b]	+0.07±0.41	+0.31±1.06	+0.03±0.16	+0.13±0.66
Positive End-Expiratory Pressure (PEEP)	+0.0±0.0	+0.02±0.16	+0.00±0.24	-0.03±0.16	+0.0±0.19
Mean Airway Pressure (P_{AW})	-0.1±1.2	+0.1±0.7	-0.1±1.4	+0.0±1.3	+0.0±1.2

a. Shown is the mean± s.d. of the change from baseline for each parameter, measured after 30 minutes.
b. Acute effects measured at baseline and after 20 minutes.
c. Acute effects measured at baseline and after 30 minutes.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.
e. Statistics calculated using Wilcoxon Rank Test comparing first baseline value to 20 minute value.
f. Statistics calculated using Wilcoxon Rank Test comparing second baseline value to 20 minute value.

The effect of I-NO on cardiac output, pulmonary vascular resistance, systemic vascular resistance, or reduction in the right-to-left shunting of blood from pulmonary hypertension were not examined in any of the trials.

5. Summary of physiological effects of I-NO

The most striking, and consistent effect of I-NO is to improve oxygenation in a fraction of the subjects exposed. In all three trials, there was an acute improvement in oxygenation which was highly significant. In the INO-01/ -02 trial, this improvement persisted beyond the initial measurement, compared with controls.

There was a nonsignficant decrease in both pCO_2 and mean systemic blood pressure acutely following I-NO administration. No long-term effect of I-NO on blood pressure, pH, or pCO_2 was seen in the INO-01/ -0_2 trial. No acute or chronic effect of I-NO on the pulmonary airway pressures was detected. No information about the pulmonary and systemic vascular resistance, or the reversal of the right-to-left shunting from pulmonary hypertension was obtained in these trials.

7.0.4 Success of trials in meeting secondary efficacy endpoints: demonstrating a clinical benefit for I-NO

Each of the three efficacy trials measured several other endpoints, either as part of secondary endpoints or as part of the safety measurements, which could be used to argue a clinical benefit. A summary of these potential benefits is below, along with the efficacy data that was collected for each benefit. Overall, no significant beneficial effect of I-NO was detected for any of the listed endpoints.

1. Duration of supplemental O_2 and use of supplemental O_2 at time of discharge

Table 7.0.4.1 Incidence of use of supplemental O_2 at time of discharge in the NINOS, INO-01/ -02 and /-03 and INOSG trials[a].

Use of O_2 at time of discharge	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value[b]
NINOS	15/100 (15%)		14/98 (14%)			0.89
INO-01/ -02 and /-03	6/41 (15%)	9/45 (20%)	4/43 (9%)	7/39 (18%)	20/127 (16%)	0.51
INOSG[b]	4/21 (19%)			1/27 (4%)		0.19

a. Data from NDA, volume 2.26 appendix 16.2.2.21, and 2.31 data listing 16.5. Data shown as % of all subjects with data. INOSG data from NDA volume 2.16, Appendix 16.2.7.

b. p values calculated using unadjusted chi-square.

Table 7.0.4.2 Duration of supplemental O_2 therapy in the INOSG trial and INO-01/ -02 trials (no data available from the NINOS trial).

Duration of supplemental O_2 (days)	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value[a]
NINOS	NA[a]					
INOSG[b]	19±21			11±6		0.066
INO-01/ -02	6±7	5±5	5±3	6±8	5±6	0.47

a. NA = not available

b. Excludes one infant in the control group reportedly required O_2 for 445 days.

The use of O_2 after discharge is discussed in section 6.0.1.13.3. No control infant required O_2 after discharge, while 12 of the infants who received I-NO (13% of the children with available data) required O_2 after discharge.

2. Duration of mechanical ventilation

Table 7.0.4.3 Duration of mechanical ventilation in the NINOS, INOSG and INO-01/ -02 trials.

Duration of mechanical ventilation	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value[b]
NINOS	11.1±13				12.3±14	0.47
INOSG	27±84			8±4		0.56
INO-01/ -02	8±5	9±7	8±5	10±10	9±7	0.94

a. NA = not available

b. p value calculated by unadjusted unpaired t test.

7.0.4 Success of trials in meeting secondary efficacy endpoints: demonstrating a clinical benefit for I-NO (cont)

3. Duration of hospitalization

Table 7.0.4.4 Duration of hospitalization in the NINOS, INOSG and INO-01/ -02 trials.

Duration of hospitalization	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value
NINOS[a]	29.5±23			36.4±45		0.17
INOSG	42±91			20±8		0.24
INO-01/ -02	26±20	22±11	21±10	24±12	24±11	0.45

a. NA = not available

4. Deaths

As shown in table 7.0.2.5 above, no difference in the rate of death between the control and I-NO groups was detected in any of the submitted trials, or in the combined population.

There were also several adverse events, which, if they occurred with less frequency in the I-NO group, could be taken as supportive of a beneficial clinical effect of I-NO. A list of these events, followed prospectively in one or more clinical trials, includes:

1) Air Leak Syndrome (ALS);
2) Bronchopulmonary dysplasia;
3) Chronic lung disease, including lung disease during the 1 year after the initial discharge from the hospital;
4) Reactive airways disease;
5) Abnormal neurological development, including neurologic development 1 year after the initial discharge from the hospital;
6) Seizures;
and 7) Clinically significant bleeding, including interventricular and intracranial hemorrhage.

The incidence of these adverse events is discussed in detail in section 8.1 and 8.2 below (Integrated Review of Safety and Review of Systems). In short, no beneficial effect of I-NO on any of these outcomes was detected in either of the large trials (NINOS and INO-01/ -02). Instead, there is a possible association between I-NO administration and increased incidence of pulmonary adverse events. In the INOSG trial, the subjects who received I-NO demonstrated a trend towards fewer days of supplemental oxygen (see 6.0.2.15 for my discussion).

7.0.5 Clinical effect of I-NO from the secondary data sources

Almost all of the published data on I-NO, with the exception of the papers reporting the NINOS and INOSG trial results, come from single centers administering I-NO in unblinded, uncontrolled fashion. For efficacy results, then, the studies are under-powered to detect a significant impact of I-NO on clinically relevant endpoints. Improvements in oxygenation have been reported in almost all trials. The percentage of infants who had such an improvement with I-NO has varied from 44% (30) to nearly 100% depending on the study. In a review of the available trials on I-NO, one group estimated that 59% of neonates have an initial improvment in oxygenation, and that 60% of these infants have a sustained response (22).

In regard to the use of ECMO, one randomized, controlled, open-label trial failed to demonstrate a reduction in the use of ECMO in subjects recieveing I-NO (21). This trial enrolled similar numbers of subjects as in the INOSG trial (23 control, 26 I-NO). In a trial using historical controls (16 total subjects), a small decrease in ECMO following I-NO was suggested (36), while another using a cross-over design (17 total subjects) found no effect of I-NO on ECMO use (30). Another investigator, in an unblinded study, estimated that 'NO apparently cut out a 15% segment from the patient group' (30 total subjects) who would otherwise have received ECMO (24).

The death rates in each of the trials was low, so no statement of an I-NO effect can be inferred from these papers. No other clinical benefits of I-NO have been demonstrated from any of the published trials.

7.5 Summary of Efficacy Review

1. I-NO administration is associated with an acute and durable increase in PaO_2. This effect of I-NO is dose-related between 5 and 80 ppm.

2. I-NO administration in the NINOS trials was associated with a significant decrease in the rate of initiation of ECMO, compared with blinded control subjects. In two other trials (INOSG and INO-01/ -02), a decrease in ECMO use was also suggested either from post-hoc analysis or from non-significant trends towards reduction. In one trial, there was evidence that the response to I-NO may depend on the underlying disease causing respiratory failure. Subjects with respiratory failure due to idiopathic PPHN and pneumonia greater decrease in the incidence of death and/or ECMO following I-NO than did subjects with MAS or RDS-associated respiratory failure. In the literature, one open-label, controlled, randomized clinical trial, of the same overall size as the INOSG trial, detected no effect of I-NO on ECMO use (21).

3. No beneficial effect of I-NO on any clinical endpoint other than reduction in the initiation of ECMO was demonstrated, or even strongly suggested, in the submitted trial data.

The reduction in the initiation of ECMO is the only durable clinical endpoint for which evidence exists for an effect of I-NO. That avoidance of ECMO is a clinically beneficial endpoint rests on the following arguments. First, ECMO is an invasive procedure, requiring highly-skilled individuals and in some cases transport of the infant to larger medical centers. ECMO administration requires systemic heparinization, and has in the past meant the loss of a carotid artery and/or internal jugular vein following the procedure. The avoidance of this procedure, then, will mean avoiding the following 'harms', discussed in turn.

1) pain and discomfort of the procedure

This risk is valid, and should be absolutely avoided. Weighing the additional pain and discomfort caused by the addition of ECMO to an infant who is already paralyzed and sedated on a ventilator is an impossible task, but is certainly less than for a conscious subject.

2) risk of heparinization, including cerebral bleeding

If this risk is appreciable, the reduction in the use of ECMO in the I-NO subjects might be expected to translate into fewer bleeding events. No such difference was noted, or even suggested from the data, although it could be argued that this is related to the small number of events. (continued on next page)

3) loss of carotid artery on one side.

This risk is increasingly of historical concern. Speaking with Dr. Ehrenkranz and other neonatologists, it is clear that veno-venous ECMO is increasingly performed, which does not require the ligature of the carotid artery. Additionally, when the carotid is used, vascular surgeons are now repairing the carotid, rather than ligating it.

4) risk of infection during ECMO administration from catheter

None of the trials measured these infections, although they should have been capture as adverse events in the INO-01/ -02 trial (there were none reported). Any catheter will be associated with some increased risk of infection. For a catheter such as the one dedicated for ECMO, the rates are quite low, much lower than peripheral IVs which are used for more than one type of infusion. Additionally, these catheters are used in an intensive care setting, and are manipulated only by highly-trained individuals.

5) risk of transport of an infant from a small facility to a larger facility where ECMO is performed.

This argument cuts both ways. If I-NO is of clear benefit, and reduces the need for transport for ECMO with its attendant risks, that would be a clear benefit for I-NO. If, however, the effect of I-NO is to delay the transfer of infants for ECMO, this delay may in fact be dangerous to the infants who receive I-NO, do not get immediately transported, and then get progressively worse and are transported in even more unstable conditions. There is also a hypothetical risk of I-NO being administered in centers without ECMO, of false reliance on I-NO in settings where the infant is declining overall, while their PaO_2 continues to be adequate due to the effects of I-NO. By the time of transfer, these infants could also be dangerously unstable, much worse than if they had been transported earlier.

The conclusion: while there are several hypothetical benefits to avoiding ECMO, all of them plausible, the current standard clinical practice has lessened the risk of the most obvious cost (loss of a carotid artery). With regards to increased bleeding due to systemic heparinization, none was detected in the three reported trials. Some of this, of course, may be related to low incidence rates of such bleeding, and the limited number of subjects in the trials. Overall, however, it is fair to say that the benefit of avoiding ECMO is not so overwhelming as to be a sufficient, single reason for approval. The use of ECMO has been shown to be life-saving in at least one controlled trial. Of note, this effect was demonstrated in only 185 infants (93 ECMO, 92 control). To avoid such therapy, the replacement therapy (I-NO in this case), must also demonstrate clear clinical efficacy. If I-NO is truly beneficial, it is reasonable to expect other clinical markers of improved outcome in the I-NO group. No such improvements were detected in any of the relevant clinical markers listed above.

7.5 Summary of Efficacy Review (cont)

Worse, there is an alternative explanation for decreased use of ECMO following I-NO adminstration. The criteria set forth in the NINOS trial to guide the use of ECMO emphasize oxygenation. In fact, an infant could be transferred to ECMO for OI>40 on two ABGs separated by at least 30 minutes (see NINOS review, section 6.0.1.3). Since the infants in the NINOS trial started with a baseline OI of 45 in control and 43 in the I-NO group, a failure to improve their OI following the first 30 minutes of exposure to study gas was sufficient to meet the criteria for transfer for ECMO. This was also the most common stated reason for transfer to ECMO in the NINOS trial (see table 6.0.1.12.2d.4). Further, the data show that of I-NO lowers OI acutely an average of 14 in the NINOS trial, 16 in the INOSG trial, and 4.6 to 7.4 in the INO-01/ -02 trial (see table 7.0.3.1 above). In this alternate interpretation of the efficacy data, the effect of I-NO was to cause an acute and durable improvement in the oxygenation of the infant. The effect of this in the NINOS trial was to defer the use of ECMO in otherwise ill subjects until the other separate therapies (i.e., HFOV/HFJV, surfactant, alkalinization) utilized in conjunction with I-NO have their salutary effect. In short, that I-NO 'turns babies pink,' reassuring the physician, and slowing their transfer of ECMO until such time that it is no longer needed. No benefit with regards to any of the hard-endpoint outcomes listed above were demonstrated for I-NO in any of the trials, or suggested from the available published literature. Thus, the benefit of I-NO is to avoid ECMO, primarily through increasing the comfort level of the attending physicians, rather than on any demonstrable salutary effect on the critically ill infant beyond an increase in oxygenation.

On the balance, then, the evidence that the use of ECMO is reduced by the use of I-NO is persuasive. The evidence however, suggests that this reduction is not associated with any other detectable clinical benefit. Further, the reduction in the use of ECMO may be a reflection of the effect of I-NO to improve oxygenation, rather than an independent clinical benefit. In such a construct, this less than overwhelming clinical benefit of I-NO must be weighed against any adverse effects associated with the use of I-NO. The next sections review the safety data concerning the administration of I-NO.

8.0 Integrated Review of Safety

8.0.1 Subsections of the Integrated Safety Review

The overall safety review will be preceded by section 8.0.1, a discussion of the safety database, and the methods used.

Following this, the safety review will be presented in section 8.1, grouped into the following sections:

1) subject deaths;
2) subjects who had serious adverse events;
3) subjects who dropped out of the studies;
4) adverse events which altered the therapy of subjects, including dropouts due to adverse events;
5) all recorded adverse events
6) laboratory findings;
7) vital signs; and
8) special studies, including tolerance, withdrawal and overdose.

In section 8.2, a review of the available safety data by body system will be performed. This will draw on the primary data presented in section 8.1. Additionally, relevant secondary data sources will be cited from section 5.2. In sections 8.2, conclusions will be made about the association between I-NO and individual adverse events.

Finally, section 8.3 will summarize the key adverse events identified in the NDA database.

8.0.2 Source Materials for the Integrated Safety Review

Safety data from the NDA will serve as the primary source of data for the safety review. It's important, however, to understand that the quantity and quality of the safety data collected in each of the studies is quite variable. The table below summarizes the safety data collected for each of the four trials. Two trials (INO-01/ -02 and -03) collected routine adverse events and clinical laboratory values. The INO-03 trial had 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) and laboratory values.

Table 8.0.2.1 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	Vital Signs	Case Report Forms[c]	Long-term Follow-up
NINOS	Y	N	Y	Y	Y	N	N[b]	N[c]	Pending
INOSG	Y	N	Y	N	Partial	N	Y	N[c]	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.
b. NINOS did not collect vital signs after exposure to study gas.
c. The NINOS and INOSG trials submitted summary outcome data-sheets for all (NINOS) or some (INOSG) of their subjects.

8.0.3 Extent of Subject Exposure to Study Gas

Four trials of I-NO were submitted as part of the NDA. The subjects exposed to each concentration of I-NO are presented below in table 8.0.1. In the NINOS trial, four subjects in the control group and 1 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 gas were administered I-NO, and 1 subject randomized to I-NO received control gas. These subjects have been discussed separately in section 6.0.1.12.3a above. The INO-03 trial enrolled only 14 subjects before being stopped, and so represents a small fraction of the total safety database.

Table 8.0.3.1 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	50	1	55	2	120
INOSG[b]	28					30		30
INO-01/02	41	41		36		37		114
INO-03	0	4		8		2		14
Total	179	42	1	100	1	124	2	278

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.

8.0.3 Extent of Subject Exposure to Study Gas (cont)

Because the duration of exposure was not normally distributed, the table below shows the median duration of exposure for each of the trials.

Table 8.0.3.2 Median duration of exposure to treatment gas (control gas or I-NO) from NINOS, INOSG, INO-01/ -02 and INO-03 trials[a].

Study	Control Group	I-NO Group
NINOS	21 hours	71 hours
INOSG	20 minutes	48 hours
INO-01/ -02	34 hours	38 hours
INO-03	No control group	91.2 hours

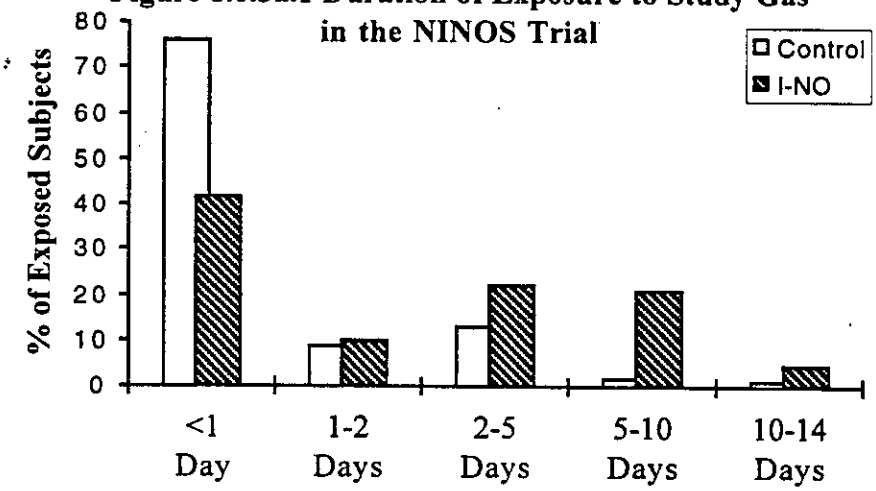
a. From NDA volume 2.50, page 339210.

8.0.3a Extent of Subject Exposure to Study Gas in the NINOS trial

The safety database for the NINOS trial consists of 120 subjects who received I-NO and 110 subjects who received control gas. As noted above, while re-trial of study gas was allowed for 'non-responder' infants, only 10 were actually retried (6/70 in the placebo non-responder group (8%) and 4/29 in the I-NO group (14%)).

The duration on study gas was 21±43 hours in the control group and 71±79 in the I-NO group ($p<0.001$). The distribution of exposure is shown in figure 6.0.1.13.2.1 below.

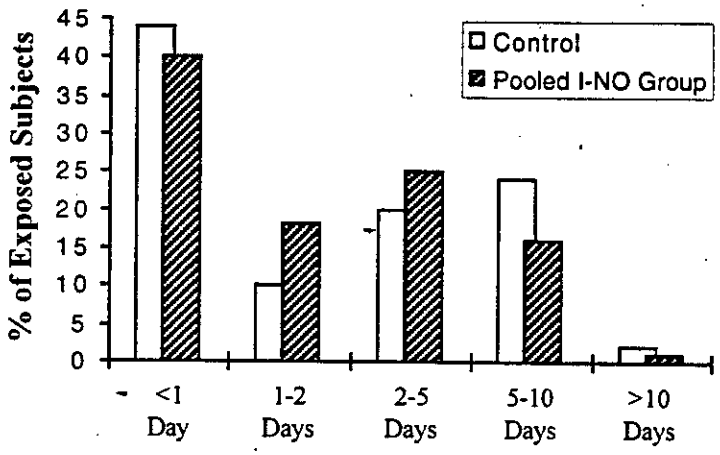
Figure 8.0.3a.1 Duration of Exposure to Study Gas in the NINOS Trial



8.0.3b Duration of Exposure in the INO-01/ -02 Trial

The safety database for the INO-01/ -02 trial consists of 41 subjects who received control gas, and 114 who received I-NO (5-80 ppm). The mean duration of exposure to the study gas was 34 hours in the control group, and 38 in the combined I-NO group. The distribution of exposure is shown in figure 8.0.3.2.1 below.

Figure 8.0.3b.1 Duration of Exposure to Study Gas in the INO-O1/ -01 Trial



Little difference between the two groups in terms of exposure time to study gas can be discerned from the pooled I-NO data shown above. Examining the exposure time for the 3 I-NO doses, however, the subjects in the 80 ppm group who were ultimately weaned successfully received I-NO for less time when compared with 5 and 20 ppm. This decrease was not significant. Overall, 54% of the control infants and 55% of the I-NO infants met weaning criteria and were successfully weaned in the INO-01/ -02 trial.

Table 8.0.3b.2 Mean duration of exposure to treatment gas (either N_2 or I-NO) for the subjects in the INO-01/ -02 trial who were ultimately weaned successfully[a].

INO-01/ -02	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Exposure (hours)	n=18 108±69	n=22 95±66	n=19 72±49	n=10 65±56	n=51 81±59

a. Data from NDA volume 2.17, table 28.

8.0.3c Duration of Exposure in the INOSG Trial

No data is available on the duration of exposure to study gas in the INOSG trial beyond the report of the mean data by the sponsor (table 8.0.3.2). If the initial treatment with study gas was successful, subjects remained on study gas as long as their OI remained <40, and weaning was attempted twice per day.

8.0.3d Duration of Exposure in the INO-03 Trial

The extremely small numbers of subjects in INO-03 preclude any firm statements about the relative duration of I-NO exposure in the three treatment groups.

Table 8.0.3d.1 Median duration of exposure to I-NO (hours) for the subjects in the INO-03 trial.

INO-03	Control Group	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Exposure (hours)	No subjects	n=4 54	n=8 109	n=2 59	n=14 91

8.0.4 General Methodologies Used for Safety Review

Four aspects of the safety database place restrictions on the scope of this review and data interpretation.

1) The ability to detect unanticipated adverse events is limited by the small number of subjects with collected data for many of the adverse events. This is especially critical for the overall adverse events and the laboratory values, which were only collected in the INO-01/ -02 trial (41 controls and 114 I-NO subjects). The numbers limit the power of the safety review to detecting one occurrence of an adverse event that occurs at a 2% event rate, with a 95% confidence. The small patient numbers limit the ability to detect differences in the incidence rates between the control and I-NO groups for infrequent and rare adverse events. This limits, first, the ability to detect an uncommon or rare adverse event, and, second, to associate its occurrence with the administration of I-NO. Likewise, sub-group, dose-dependence, or duration of exposure analyses will be difficult to interpret for these infrequent adverse events. In the absence of larger numbers of subjects, conclusions will be drawn from the available data, recognizing that meaningful statistical analysis of the differences is impossible. Where inadequate numbers of subjects exist even to consider such an analysis, that adverse event will be labeled as having inadequate data.

2) Some adverse events of clinical interest had no safety data collected during any of the four clinical trials. These events are related to laboratory measurements, such as changes in coagulation parameters, and other measures normally performed during trials (urinalyses, ECGs).

2) Details of individual cases are limited for both the trials performed by academics (NINOS and INOSG), where only case summaries are available. This limits the specific information regarding events which occurred in those trials obtainable through examination of the case report forms. In the INO-01/ -02 trial, case report forms and follow-up information were requested for individuals with any adverse events which did not resolve before discharge, for unresolved adverse events and abnormal labs, and for specific adverse events of particular clinical relevance. Information available at the time of the NDA withdrawal is included in the review (9.16.97).

3) Identification of serious adverse events, and adverse events leading to withdrawal from the trial were similarly not routinely performed in the NINOS and INOSG trials. These data will be drawn primarily from the INO-01/ -02 and /-03 trials. The conclusions drawn from these data will be extrapolated to include all infants with hypoxemic respiratory failure, despite the differences in the baseline characteristics of the four trials. In particular, the INO-01/ -02 trial differed from the NINOS and INOSG both in terms of the severity of the infants illness at baseline, and with regards to specifics of their clinical conditions (i.e., INO-01/ -02 required echocardiographic evidence of PPHN, but excluded infants with prior surfactant and HFOV/HFJV therapies). The limitations of such an extrapolation are obvious.

4) In the INO-01/ -02 trial, adverse events were collected by two sets of reviewers. First, all adverse events which were identified by the individual investigators were collected at the time that they occurred. These adverse events included all 'unexpected' adverse events (i.e., agranulocytosis, hypomagnesemia). Certain adverse events (i.e., pneumothorax, seizures) were also collated at the end of the hospitalization (or after death) by data managers using the hospital record. For certain adverse events, then, two sets of incidences were collected, which may be different. Of the two, the adverse events identified by the investigators are given more weight in this review, as they were felt to have altered therapy.

The safety review, then, will use two different data-sets from the trials submitted in the NDA to address two large sets of safety concerns.

First, the incidence of death, overall drop-outs, and certain pre-defined adverse events was examined in most or all of the trials. Given this larger number of exposed subjects for whom we have information for these events, more through sub-group analysis can be performed. These events will be analyzed separately for each trial and then, where possible, combined for analysis.

Second, for the majority of the unanticipated adverse events, including serious adverse events, lab abnormalities, and drop-outs due to adverse events, the database will be comprised primarily of INO-01/ -02 subjects, along with the 14 subjects in the INO-03. For these adverse events, analysis will focus on identifying events which might be linked to the use of I-NO, and less sub-group analysis will be possible. This information will be integrated with data from case report forms, and from data from secondary sources in section 8.2. The search strategies for the laboratory adverse events are discussed further in section 8.1.6.2 below.

With these caveats, then, the mechanism used for review of each type of adverse event was similar. First, the database was identified, and the number of subjects with data and the duration of exposure summarized. Next, any adverse events identified by the sponsor or the individual investigators were examined. Details of the search mechanism, and the sources used to identify adverse events are found in section 8.1.5.1.2 below (Identifying Key Adverse Events). Next, the rate a given adverse event occurred in the controls was compared with the I-NO group. Any event which occurs exclusively in the I-NO group, or ≥2X more frequently, was scrutinized, with the results detailed in section 8.2. Details of the safety data in each trial, and the 8.1.5.1 Approach to Eliciting Adverse Events in the Development Program

8.0.4 General Methodologies Used for Safety Review (cont)

Next, sub-group analyses were performed looking for individual subjects with 'outlier' events. This was particularly important for the lab values and for certain adverse events (withdrawal, elevated methemoglobin and NO_2 levels, death). For these analyses, the data from individual subjects were examined by this reviewer. The infants who had identified Serious Adverse Events, and all subject deaths, will also be integrated in to each relevant body system review. Case report forms from individual subjects were requested from the sponsor (only available for the INO-01/ -02 and /-03 trials) where relevant.

Where sufficient numbers exist, sub-group analyses by race or sex were also performed.

Finally, the database was scrutinized for potential adverse events raised by other investigators in publications. These potential adverse events are detailed in section 5.2 (Secondary Source Data). Where relevant, these results are included in section 8.2.

8.1 Background Data for the Safety Review

8.1.1 Deaths

The table below shows the mortality rate in each of the trials. In the NINOS trial, the intention to treat group is shown as well as the number of deaths that occurred in the trial, grouped according to the gas actually received.

Table 8.1.1.1 Incidence of death in the NINOS, INOSG, INO-01/ -02 and INO-03 studies.

Study	Control group	I-NO group	p value[d]
NINOS (0-120 days)[c]	20/121 (16.5%)	16/114 (14.0%)	0.60
NINOS (0-120 days)[b]	17/112 (15.1%)	17/118 (14.4%)	0.87
INOSG (0-445 days)	3/28 (10.7%)	2/30 (6.7%)	0.70
INO-01/ -02 (0-28 day)	1/41 (2.4%)	9/113 (8%)	0.29
INO-01/ -02 (0-1 yr)	2/41 (4.9%)	10/113 (8.8%)	0.42
INO-03 (0-28 days)	No control group	0/14 (0%)	N/A
Total[a,e]	24/190 (12.6%)	27/271 (9.9%)	0.43
Total[f]	22/181 (12.1%)	29/275 (10.5%)	0.28

a. The comparability of the incidence rates between the trials is limited by the varying length of follow-up for each trial (shown in parenthesis).

b. Grouped from the NINOS subjects who actually received study gas, according to the gas actually received.

c. Grouped from the NINOS ITT population.

d. p value calculated using unadjusted chi-square.

e. Incidence of death calculated from the ITT NINOS population, the INOSG population, the INO-01/ -02 population 0-28 days, and the INO-03 trial population.

f. Incidence of death calculated from the 'gas received' NINOS population, the INOSG population, the INO-01/ -02 0-1 year population, and the INO-01/ -02 trial population.

8.1.1 Deaths (cont)

Table 8.1.1.2 Deaths in the control groups from NINOS, INOSG, INO-01/ -02, and INO-03 studies[a,d].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b,c]
NINOS				
	3-A05	Yes	12	Multi-organ failure, withdrawal of support
	3-A08	No	2	Severe hypoxia
	7-01	Yes	6	Severe intracranial hemorrhage
	10-A05	No	2	Refractory pulmonary hypertension
	12-A13	Yes	9	Severe intracranial hemorrhage, withdrawal of support
	14-A02	Yes	20	Suspected sepsis/infection
	15-A08	Yes	12	Alveolar-capillary dysplasia
	51-A11	Yes	16	Alveolar-capillary dysplasia
	52-A02	No	2	Suspected sepsis/infection
	52-A09	No	3	Left ventricular failure
	52-A14	No	3	RDS
	54-A03	No	1	Suspected sepsis/infection
	54-A14	No	5	Severe pulmonary hypertension
	55-A05	Yes	14	Polycystic kidneys
	56-A08	Yes	1	Proven sepsis/infection
	57-A01	No	2	Pulmonary hypoplasia
	58-A03	No	1	Proven sepsis/infection
	59-A03	No	5	Suspected sepsis/infection
	59-A08	Yes	3	Severe intracranial hemorrhage
	60-A02	No	43	Broncho-pulmonary dysplasia
INOSG				
	Buf-8	Yes		Heart failure Withdrawal of support
	CHOP-S2	Yes		Anoxic brain injury Withdrawal of support
	YALE-4	No		N/A
INO-01/ -02				
	02-15005	No	5	PPHN Multiple Chromosomal Anomalies Withdrawal of support
INO-03				
	No deaths			

a. Any death prior to 120 days is included in the NINOS data. No specific information regarding the deaths in the INOSG trial is available.
b. Description of individual deaths is derived from company-provided narrative. Where possible, individual case report forms were scrutinized by this reviewer as well. This was done for all deaths in the INO-01/ -02 trial.
c. Full narrative summaries of each death are included below, where available.

8.1.1 Deaths (cont)

Table 8.1.1.3 Deaths in the I-NO groups from NINOS, INOSG, INO-01/ -02 and INO-03 studies[a].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b,c]
NINOS[e]				
	3-A04	No	20	Respiratory failure
	5-A07	Yes	16	Severe CNS ischemia
	5-A14	No	4	Suspected sepsis/infection
	5-A20	Yes	1	RDS
	5-A25	Yes	60	'Thrombi' Withdrawal of support
	12-A11	Yes	15	Alveolar-capillary dysplasia
	15-A09	Yes	5	Proven sepsis/infection
	51-A08	Yes	18	Pulmonary lymphangiectasia
	52-A04	No	2	Meconium aspiration
	51-A06	Yes	5	
	55-A09	No	10	Withdrawal of support
	55-A21	Yes	136	Proven sepsis/infection
	56-A14	No	1	Suspected sepsis/infection
	57-A02	No	1	Proven sepsis/infection
	58-A01	No	1	Surgical death
	59-A02	No	8	RDS
INOSG				
	Buf-17	Yes	N/A	N/A
	UT@D-1	No	N/A	Pneumomediastinum
INO-01/ -02				
	01-11012	No	21	Possible sepsis Progressive hypoxia Recurrent pneumothoraces Withdrawal of support
	01-17003	Yes	8	Meconium aspiration, Pseudomonas sepsis
	01-1006	No	5	Sepsis, hypotension, bradycardia
	01-03023	No	11	Meconium aspiration, seizures, Gross neurologic deficits
	01-03025	No	6	PPHN, renal failure, Encephalopathy, pneumothorax
	01-11005	No	3	Meconium aspiration, Pneumothorax, cardiopulmonary arrest
	01-03026	Yes	17	PPHN, alveolar capillary dysplasia
	01-06006	No	17	Perinatal hypoxia, PPHN, acidosis, recurrent pneumothoraces
	01-11011	No	3	Idiopathic PPHN, Recurrent pneumothoraces and pneumopericardium
INO-03				
	No deaths			

a. Any death prior to 120 days is included. No specific information regarding the deaths in the INOSG trial is available.

b. Description of individual deaths is derived from company-provided narrative. Where possible, individual case report forms were scrutinized by this reviewer as well. This was done for all deaths in the INO-01/ -02 trial.

c. Full narrative summaries of each death are included below.

d. No deaths occurred in the first 28 days of the INO-03 trial.

e. NINOS study subjects identified by center #-patient # (e.g., 05-A04).

In the INO-01/ -02 trial, subjects were re-evaluated after 1 year to ascertain vital status, as well as to assess the incidence of other adverse events (discussed below). The table below summarizes the data on the subjects with available data.

8.1.1 Deaths (cont)

Table 8.1.1.4 Deaths from the INO-01/ -02 1 year follow-up data[a,d].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b,c]
INO-01/ -02 Control				
	02-14004	No	90	PPHN, SIDS death after discharge
I-NO 20 ppm				
	01-11015	No	32	Idiopathic PPHN, pneumothoraces, sepsis

a. Any death after one month.

b. Description of individual deaths is derived from company-provided narrative. Where possible, individual case report forms were scrutinized by this reviewer as well. This was done for all deaths in the INO-01/ -02 trial.

c. Full narrative summaries of each death are included below.

8.1.1.1 Narratives of individual subject deaths. Note: no individual case narratives are available for either the NINOS or the INOSG trials.

Given the small number of deaths for which we have narrative information and case report forms available, these narratives will be included here, rather than placed in the appendix. A discussion of the individual deaths, and their links to specific adverse events and I-NO administration, will be performed in the appropriate sections of 8.2.

8.1.1.1a Deaths in Control Subjects from INO-01/ -02 .

1. Subject 02-15005: a 2.9 kg white male, born after a 39 week gestation to a mother whose pregnancy was complicated by first and second trimester bleeding, urinary tract infection, anemia and oligohydramnios. The patient had Apgar scores of 6 and 7, and developed PPHN. The infant had dysmorphic features, and his karyotype was revealed to be 49, XXXXY. Further w/u revealed ventricular septal defects, renal failure, hypotonia and hydrocephalus. He was started on treatment gas (placebo), but oxygenation did not improve, and he was removed after 8 hrs and 20 minutes. Given the patient's poor prognosis, it was decided to remove aggressive life support, and he died of severe hypoxemia 5 days after initiation of study gas.

8.1.1.1b Deaths in I-NO Subjects from INO-01/ -02 .

1) Subject 01-11012: a 3.2 kg white male, born after a 40 week gestation to a mother whose pregnancy was complicated by hypertension during the last 2 months. The patient had Apgar scores of 7 and 9, and developed PPHN, possibly due to sepsis. He was started on treatment gas (I-NO 5 ppm), but was discontinued after 10 hours 20 minutes because of persistent hypoxemia (PaO_2 baseline 56, 30 minute value, 57). He subsequently received HFOV, HFJV, and surfactant. On approximately day 20, the patient suffered a right pneumothorax, and had progressive hypoxemia. A decision was made to withdraw therapy, and the subject died 21 days after initiation of study gas.

2) Subject 01-17003: a 4.5 kg white male, born after a 42 week gestation by emergency cesarean section for fetal distress precipitated by an umbilical cord knot, to a mother whose pregnancy was complicated by hypothyroidism requiring medication. The patient had Apgars of 4 and 7 and developed PPHN from meconium aspiration. He was started on treatment gas (I-NO 5 ppm) with little acute effect (PaO_2 baseline 63, 30 minute value, 69), and study gas was withdrawn 25 hours later because the principle investigator felt the patient's oxygenation had not improved. He subsequently received high-frequency ventilation and surfactant, with improvement. On day 7, the patient developed *Pseudomonas* pneumonia and shock. He was started on ECMO but died the same day.

3) Subject 01-01006: a 3.7 kg white male, born after 38 weeks gestation to a mother whose pregnancy was uncomplicated. The patient had Apgar scores of 8 and 9, and developed PPHN from presumed sepsis. He was started on treatment gas (I-NO 20 ppm) with initial improvement in oxygenation (PaO_2 baseline 47, 30 minute value 202), followed by a return of hypoxemia within 36 hours despite continued I-NO. The patient continued to require maximal vasopressors for blood pressure support. His blood cultures grew group B *Streptococci*, and the patient ultimately died after progressive hypotension, bradycardia, and a decision to withhold further support.

8.1.1 Deaths (cont)

4) Subject 01-03023: a 3.8 kg Hispanic male, born after 41 weeks of gestation by emergency cesarean section for fetal distress. The patient's Apgars were 1 and 1, and heavy meconium staining was noted. The infant was intubated immediately, and seizures refractory to therapy developed. PPHN from meconium aspiration developed and he was started on study gas (I-NO 20 ppm), with no immediate change in PaO_2, followed by a gradual improvement in respiratory status over several hours. He was weaned from I-NO after 5 days, but the patient continued to have severe neurologic impairment, and aggressive therapy was withdrawn. The patient died 11 days after initiation of I-NO.

5) Subject 01-03025: a 4.1 kg white male, born after 38 weeks of gestation by cesarean section for failure to progress during delivery, to a woman who received limited prenatal care. His Apgar scores were 8 and 8, and he developed PPHN and RDS. He was started on treatment gas (I-NO 20 ppm) with no acute increase in PaO_2 (43 at baseline to 42 after 30 minutes). He showed gradual improvement, and was continued on I-NO for 104 hours, after which he was weaned successfully. Evaluation of the infant revealed severe periventricular leukomalacia and a burst pattern on EEG, and persistent renal failure. The decision was made to withdraw therapy, and the infant died 6 days after starting I-NO.

6) Subject 01-11005: a 3.7 kg black female, born after 42 weeks of gestation by emergency cesarean section for fetal distress to a mother whose pregnancy was complicated by oligohydramnios. The patient's Apgars were 1 and 1, and PPHN developed as the result of meconium aspiration. She was started on treatment gas (I-NO 20 ppm) with poor response (PaO_2 78 at baseline to 94 after 30 minutes). Oxygenation gradually declined until she was withdrawn from I-NO after 56 hours of therapy. HFOV was used without improvement, and the patient died after developing a pneumothorax.

7) Subject 01-03026: a 2.8 kg Filipino female, born after 40 weeks of gestation to a mother whose pregnancy was complicated by an asymptomatic heart murmur. The patient had Apgars of 8 and 9, and developed PPHN. She was started on study gas (I-NO 80 ppm) with a dramatic, transient increase in PaO_2 (from 41 at baseline to 321 after 30 minutes to 29 after 1 hour). The patient was withdrawn from I-NO after 33 hours. The patient then received HFOV and ECMO, complicated by bleeding at the catheter site, platelet consumption, abnormal LFTs (pre-dating I-NO therapy) and worsening hypoxemia. She became bradycardic and died 12 days after starting therapy. Autopsy revealed misalignment of the pulmonary veins with alveolar capillary dysplasia.

8) Subject 01-06006: a 4.1 kg black female was born after 42 weeks of gestation by difficult vaginal delivery including shoulder dystocia and a nuchal cord that had to be cut and clamped 4 minutes before delivery. The mother had gestational diabetes. The patient's Apgars were 1 and 6 and she required resuscitation in the delivery room. She developed PPHN and was started on study gas (I-NO 80 ppm). Due to methemoglobinemia (>7%) she was weaned to 32 ppm, and her PaO_2 remained between 60 and 100 (baseline 60) for 5 days. I-NO was discontinued after 6 days, and she was given HFOV. She developed a series of pneumothoraces, became bradycardic and progressively hypoxemic, and died 17 days after therapy started.

9) Subject 01-11011: a 4.2 kg white male, born after 40 weeks of gestation by elective cesarean section. His mother had chlamydia during the pregnancy. The patient's Apgars were 8 and 9, and developed idiopathic PPHN. He was started on treatment gas (I-NO 80 ppm), with no initial response (PaO_2 was 60 at baseline, 62 after 30 minutes). There was a small, gradual increase in PaO_2 with time, and the subject received I-NO for 60 hours, at which time he developed pneumothoraces and a pneumopericardium and died.

8.1.1.1c Narratives of deaths in the INO-01/ -02 trial during long-term follow-up

Control

1) Subject 02-14004: a 3.1 kg white male, born after 38 weeks of gestation to a mother whose pregnancy was complicated by first trimester bleeding. Patient had Apgar scores of 8 (1 minute) and 9 (5 minutes). He developed PPHN as a complication of pneumonia and sepsis. He started treatment gas (placebo), and improved, so that the gas was discontinued after three days. He was ultimately discharged, and did well up to 90 days after treatment initiation. He was found dead in his crib after being well the night before, and a diagnosis of Sudden Infant Death syndrome was made.

8.1.1.1c Narratives of deaths in the INO-01/ -02 trial during long-term follow-up (cont)

I-NO 20 ppm

2) Subject 01-11015: a 2.6 kg white male, born after a 37 week gestation, was born after an uncomplicated pregnancy. The patient's Apgars were 4 and 9, and required a chest tube placement in the delivery room for a pneumothorax. A diagnosis of PPHN from RDS was made, and the patient was started on study gas (I-NO 20 ppm) with little change in oxygenation (PaO_2 45 at baseline, 41 after 30 minutes). The patient had multiple pneumothoraces and remained hypotensive and thrombocytopenic. I-NO therapy was weaned after 140 hours. HFJV was attempted without improvement, the patient developed cystic bronchopulmonary dysplasia and *Strep. epidermidis* sepsis, anasarca, and ultimately died after a cardiac arrest 32 days after starting therapy.

8.1.1.1d Narratives of deaths in the NINOS and INOSG trials

No narratives are available for either trial.

8.1.2 Other Serious Adverse Events

Note: several adverse events were followed prospectively in the trials, which will be included in section 8.1.5 and in the discussion of individual organ systems below (these were also discussed in the section on the NINOS trial). Only those adverse events which were identified by the investigators as 'serious' are included in this section.

8.1.2.1 Serious Adverse Events from the NINOS trial

Two individual serious adverse events were identified, which are summarized in the table below.

Table 8.1.2.1.1 Serious Adverse Events from the NINOS study[a].

Study Group	Subject #	Adverse Event	Withdrawn from Study?	Outcome	Related to Drug?[b]
Control	11-A04	Excessive bleeding at ECMO cannulation site	No	Surgery stopped bleeding	No
I-NO	12-A01	Hypertensive, bradycardic, hypokalemic episode after acutely stopping I-NO (20 ppm)	No	Weaned after 89 hrs on gas, left hospital	Temporally associated

a. Data from NDA volume 2.14 and electronic datasets.
b. Per individual investigator.

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8.1.2.2 Serious Adverse Events from the INO-01/ -02 trial

A total of 24 serious adverse events were identified by the investigators in the INO-01/ -02 trial. These events, including those events which led to the death of the subject, are included in the table below. The narratives for the adverse events which resulted in death are included above. The other serious adverse events are summarized in the table and narrative below.

Table 8.1.2.2.1 Serious Adverse Events from the INO-01/ -02 study[b].

Study Group	Subject #	Adverse Event	Withdrawn from Study?	Outcome	Related to Drug[a]?
Control	01-03019	Laryngomalacia	No	Recovered	Not Related
		Hydrocephalus	No	Recovered	Not Related
	01-04001	Seizure	No	Recovered	Not Related
	02-14004	Sudden Infant Death Syndrome (SIDS)	No	Died	Not Related
	02-15004	Pneumonia	No	Recovered	Not Related
	02-15005	Pneumonia	No	Died	Not Related
I-NO 5 ppm	01-11012	Pneumothorax	No	Died	Not Related
	01-17003	Pseudomonas sepsis	No	Died	Not Related
I-NO 20 ppm	01-01006	Bradycardia	Yes	Died	Remotely Related
	01-03015	Cerebral infarct	No	Continues	Not Related
	01-03023	Perinatal asphyxia	No	Died	Not Related
	01-03025	Renal Failure	No	Sequelae	Not Related
		Encephalopathy	No	Died	Not Related
		Seizures	No	Sequelae	Not Related
		Pneumothorax	No	Sequelae	Not Related
	01-11005	Pneumothorax Pneumopericardium	No	Died	Not Related
	01-11015	Cardiopulmonary arrest	No	Died	Not Related
I-NO 80 ppm	01-01002	Conjugated hyperbilirubinemia	No	Continues	Possibly Related
	01-03026	Alveolar capillary dysplasia	No	Died	Not Related
	01-04005	Hypoxic encephalopathy	No	Sequelae	Not Related
	01-06006	Tension pneumothorax	No	Died	Not Related
	01-11011	Cardiopulmonary arrest	No	Died	Remotely Related
		Bilateral pneumothoraces	No	Died	Remotely Related
		Bradycardia	No	Died	Remotely Related
		Pneumopericardium	No	Died	Remotely Related

a. Relationship to I-NO administration per principle investigator.
b. Data from volume 2.17 and electronic datasets.

8.1.2.3 Patient narratives for dropouts from serious adverse events from INO-01/ -02

Only the subjects who with serious adverse events who survived are included in this section. The narratives for the subjects who died are in section 8.1.1.1 above.

1. Subject 01-01002, a 3.3 kg black male, developed PPHN as a result of meconium aspiration, as well as pneumomediastinum. His admission labs revealed elevated LFTs (LDH 7908, SGOT 332, Total Bilirubin 13.6) and anemia (hematocrit 26). He had an acute response to I-NO, 80 ppm (PaO_2 60 at baseline to 196 after 30 minutes). The abnormal LFTs improved on follow-up with the exception of the bilirubin, which improved but did not resolve. Subject survived without ECMO, suffered a single seizure shortly after admission, and was discharged with chronic lung disease.

2. Subject 01-03015, a 2.7 kg Hispanic female, developed PPHN possibly due to sepsis. Her admission labs revealed elevated LFTs (LDH 1185, SGOT 113, Total Bilirubin 4.7). She responded to I-NO, 80 ppm (PaO_2 at baseline 50 to 178 after 30 minutes). She was diagnosed with a 'left middle cerebral artery infarction', and the investigator felt that 'the infarct was probably caused during the period of severe R to L shunting which preceded' treatment with I-NO. The subject received I-NO for approximately 8 hours and the LFT abnormalities resolved. She did not receive ECMO and was discharged without chronic pulmonary disease.

8.1.2.3 Patient narratives for dropouts from serious adverse events from INO-01/ -02 (cont)

3. Subject 01-03019, a 3.4 kg Hispanic male, developed PPHN with meconium aspiration. His admission labs revealed elevated LFTs (LDH 1895, SGOT 155, Total Bilirubin 2.5). He had no acute response to I-NO, 80 ppm (PaO_2 from 43 at baseline to 38 after 30 minutes), and his PaO_2 did not improve over the next 12 hours of I-NO therapy and it was discontinued. His LFTs improved but did not resolve (LDH 1041, SGOT 129, Total Bilirubin 45). He developed hydrocephalus as the result of intracerebral and intraventricular hemorrhage, neonatal seizures, vocal cord paralysis and severe laryngomalacia from intubation. No further laboratory data are available. He received ECMO and HFOV, and required O_2 at time of discharge.

4. Subject 01-04001, a 3.7 kg African American male, developed PPHN with meconium staining. He had no acute response to I-NO, 80 ppm (PaO_2 64 at baseline to 64 after 30 minutes), and he received the I-NO for 36 hours. He had a seizure in the immediate post-natal period. He was deemed a treatment failure due to persistent hypoxia, received surfactant and conventional ventilation and recovered. He was discharged without ECMO or chronic lung disease.

5. Subject 01-04005, a 2.9 kg Hispanic male, developed idiopathic PPHN. His admission labs showed marked LFT abnormalities (LDH 1976, SGOT 774, Total bilirubin 4.1) and thrombocytopenia (93,000). He had no initial response to I-NO, 80 ppm (PaO_2 79 at baseline to 86 after 30 minutes) but improved thereafter. He received the I-NO for 12.5 hours before being discontinued due to elevated methemoglobin levels. The SGOT improved but did not resolve (313 after 5 days). The thrombocytopenia worsened (72,000 after 5 days). No further lab records are available. He did not receive ECMO and was discharged without chronic lung disease.

6. Subject 02-15004, a 3.6 kg white male, developed PPHN secondary to pneumonia. He had a partial response to I-NO, 80 ppm (PaO_2 41 at baseline to 54 after 30 minutes) and the subject was withdrawn from I-NO after 3 hours. No admission LFTs or chemistries are available. He was deemed a treatment failure after rapid clinical decline on I-NO. His oxygenation improved slowly after discontinuation of I-NO, and he was discharged without ECMO or chronic pulmonary disease.

8.1.2.4 Serious Adverse Events from the INOSG trial

The occurrence of all adverse events was not collected in the INOSG trial. Specific adverse events were followed prospectively, which will be incorporated into the discussion of adverse events by individual organ systems according to body system in section 8.2.

8.1.2.5 Serious Adverse Events from the INO-03 trial

Eight of the 14 subjects in INO-03 developed an adverse event, of which two were classified as 'moderately severe'. There were no serious adverse events.

8.1.3 Dropouts

Subjects in all four major trials were withdrawn from the study gas for several reasons, including if they failed to improve after study gas administration. As a result, the drop-outs in the studies most closely approximate the 'treatment failures' seen in other safety databases. The tables below summarizes the percentage of each treatment group that discontinued each trial. The NINOS group includes all randomized subjects, including the 5 subjects (4 control, 1 I-NO) who did not received study gas at all.

The first table summarizes the three trials in which data exists on subjects who were withdrawn for a variety of reasons from treatment gas (hence, classified as treatment failures).

Table 8.1.3.1 Subjects from NINOS, INO-01/ -02 and INO-03 who were withdrawn from study gas[a].

Study	Control Group	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
NINOS[b]	97/121 (80%)				60/114 (52%)
INO-01/ -02	23/41 (56%)	19/41 (46%)	17/36 (47%)	27/37 (73%)	63/114 (55%)
INO-03	No subjects	0/4 (0%)	1/8 (12.5%)	1/2 (50%)	2/14 (14%)

a. Reasons for withdrawal include: failure to maintain a PaO_2 >40 mmHg; cardiopulmonary instability; treatment side effects; methemoglobin concentrations >7%; NO_2 persistently >3 ppm.

b. The results of the NINOS trial are shown for the pooled I-NO group in this table, since it includes withdrawals from both 20 and 80 ppm I-NO.

The next table summarizes the data from all four trials on subjects who were withdrawn for 'treatment failure.' [a] The subjects are sorted according to the various definitions of 'failure' from each of the trials, which are defined in the footnotes.

8.1.3 Dropouts (cont)

Table 8.1.3.1 Subjects from NINOS, INO-01/ -02 and INO-03 who were deemed to have not responded to a given therapy[a].

Study	Control Group	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
NINOS low-flow non-responders[b]	100/121 (83%)		55/114 (49%)		
NINOS high-flow non-responders[j]	81/100 (81%)			50/55 (91%)	
INO-01/ -02 'treatment failures[c]	14/41 (34%)	11/41 (27%)	10/36 (28%)	22/37 (59%)	43/114 (38%)
INO-01/ -02 total disontinuations	23/41 (56%)	19/41 (46%)	17/36 (47%)	27/37 (73%)	63/114 (55%)
INO-01/ -02 non-responders[h]	32/40 (80%)	25/41 (61%)	20/35 (57%)	15/36 (42%)	60/118 (51%)
INOSG failure[f]	26/28 (93%)			14/30 (47%)	
INOSG non-responders[g]	28/28 (100%)			17/30 (57%)	
INO-03[i]	No subjects	0/4 (0%)	1/8 (13%)	1/2 (50%)	2/14 (14%)
INO-03 non-responders[h]	No subjects	2/4 (50%)	3/8 (39%)	1/2 (50%)	6/14 (43%)

a. The four protocols varied slightly in how they defined a failure to respond.

b. NINOS defines a full response to study gas as an increase in PaO_2>20 mmHg, and a partial response as an increase of 10-20 mmHg. This row includes those subjects who had no response to I-NO (<10 mmHg increase in PaO_2). Data from NDA volume 2.14, page 029808.

c. Treatment failure was defined as meeting any one of the following: a. PaO_2<40 mmHg at the beginning and end of a 30 minute period not due to a correctable mechanical problem; b. Mean systemic arterial pressure <35 mmHg after volume or vasopressor therapy; c. the subject died; d. the subject's methemoglobin >7% on two consecutive time points at least 30 minutes apart; or e. the subject's NO_2 level was persistently > 3 ppm for 30 minutes.

d. In addition to subject withdrawal for treatment failure, as defined in c above, subjects could be withdrawn from the INO-01/ -02 trial for other reasons: delivery device malfunction; investigator decision; and acute deterioration requiring immediate therapy (not waiting for the 30 minute gas).

e. Non-responders were to have been identified following three trials of study gas, with 3 successive failures to respond. As noted above, few subjects actually had more than one trial of study gas.

f. Success was defined in the INOSG as PaO_2>55, OI<40 and mean BP >40 mmHg after 20 minutes.

g. This is a post-hoc analysis, using the definition of success from the NINOS trial to determine the % of subjects who failed to increase their PaO_2 >20 mmHg after 20 minutes, independent of the other components of the definition of success in the INOSG trial.

h. This is a post-hoc analysis, using the definition of 'success' used in the NINOS trial. The % of subjects who failed to increase their PaO_2 by >20 mmHg after 30 minutes on study gas is tabulated.

i. INO-03 defines 'treatment failure' as a PaO_2<40 mmHg on study gas for 30 minutes.

j. NINOS high-flow includes only those infants who had either no response or a partial response to I-NO 20 ppm.

Interpretation of this table is complex, and relies on the different definitions of 'failure' from the three trials.

1. The first point is that, from the NINOS data, failure to have a response to 20 ppm I-NO made it very unlikely that an infant would respond acutely to 80 ppm (first two rows of table). Of the 55 subjects who had either a partial or no response to I-NO 20 ppm, only 6 (9%) had an increase in their PaO_2 of >20 mmHg after exposure to I-NO 80 ppm for 30 minutes.

2. For most of the analyses, exposure to I-NO lowered the % of subjects who were 'non-responders' to study gas, relative to the controls. In general, responders represent those infants who had an acute increase in oxygenation after exposure to the study gas.

a. In the NINOS trial, there was a 41% reduction in risk for non-response in the I-NO group (83 to 49%).

b. In the INO-01/ -02 trial, there was a roughly 35% reduction in risk for being a non-responder in the I-NO group (34 to 23%, 80 to 51% reductions, depending on the definition used).

c. In the INOSG trial, there was a roughly 45% reduction in risk for being a non-responder in the I-NO group (93 to 47%; 100 to 57% reductions, depending on the definition used).

d. There is no control group in the INO-03 study, so interpreting the acute response data is difficult.

3. From the INO-01/ -02 data, there appears to be a dose-response curve between I-NO concentration and acute increase in PaO_2. This was discussed more fully in the relevant section in the INO-01/ -02 review.

8.1.3.1 Overall Profile of Dropouts

This section will focus on the subjects who were considered therapeutic failures, and discontinued the study gas before they were successfully weaned. The table below summarizes the available information regarding the specific reasons individuals did not complete a given trial. Note that is some columns a given subject may have been withdrawn for more that one reason. Also note that some individuals could appear in more than one column (for instance, subjects who were withdrawn from the INO-01/ -02 trial due to elevated methemoglobin were also counted as 'treatment failures.'

Table 8.1.3.1.1 Reasons for therapeutic failures of study gas in the NINOS, INOSG, INO-01/ -02 and INO-01/ -02-03 trials[k].

Study	Discontinuation before weaning	Reasons for Discontinuation (shown as % of total discontinuations)						
		Non-responder or Treatment Failure[i]	ECMO	Consent withdrawn[e]	Death	Cardio-pulmonary Instability	Elevated MetHgb or NO2[g]	Other
NINOS								
Control	97/121 (80%)	49 (50%)	28 (29%)[a]	0	1 (1%)		0	4 (4%)
I-NO 20 ppm	60/114 (53%)	41 (67%)	29[b]	0	3 (5%)	See Note[m]	0	6 (10%)[c]
INO-01/ -02								
Control	23/41 (56%)	15 (65%)		8 (35%)		14 (61%)	0	3 (13%)
I-NO 5 ppm	19/41 (46%)	12 (63%)		8 (35%)		11 (60%)	0	1 (5%)
I-NO 20 ppm	17/36 (47%)	10 (59%)		7 (41%)		9 (53%)	0	1 (6%)
I-NO 80 ppm	27/37 (73%)	22 (82%)		5 (18%)		9 (33%)	14 (48%)[j]	2 (7%)
I-NO combined	63/114 (55%)	56 (49%)	See Note[f]	20 (32%)	See Note[f]	29 (46%)	14 (22%)	4 (6%)
INO-03								
I-NO 5 ppm	1/4 (25%)			1 (100%)			0/4 (0%)	
I-NO 20 ppm	2/8 (25%)	1 (25%)		2 (100%)		2/8(25%)	0/8 (0%)	
I-NO 80 ppm	2/2 (100%)		See Note[f]		See Note[f]	1/2 (50%)	2/2 (100%)	
INOSG[d]								
Control	26/28 (93%)		20 (77%)	No data	No data		No data	No data
I-NO 80 ppm	14/30 (47%)		12 (86%)	No data	No data		No data	No data

a. Includes two subjects who were withdrawn from study gas for transfer to another facility for ECMO.
b. Includes one subject who was withdrawn from study gas for transfer to another facility for ECMO.
c. Includes three subjects who were withdrawn after receiving 14 days of I-NO.
d. Subjects were withdrawn from study gas if they did not respond after 20 minutes with an increased PaO_2. Details of any other reasons for withdrawal are not available.
e. The consent withdrawn category includes subjects withdrawn because the investigator felt it was in the best interests of the child. Cardiopulmonary instability, elevated methemoglobin and NO_2 levels were considered as 'treatment failures' (see section 6.0.3.12.2d).
f. In the INO-01/ -02 and /-03 trials no infant was specifically withdrawn for ECMO or due to death, although the timing of the withdrawal from study gas and delivery of ECMO or the occurrence of death might be quite close.
g. Subjects were withdrawn if methemoglobin levels were >7% in the INO-01/ -02 trial or >10% in the NINOS trial.
i. Subjects were withdrawn as 'non-responder' in the NINOS trial, and as 'treatment failures' in the INO-01/ -02 trial.
j. One individual in the INO-01/ -02 had an elevated NO2 to this level. That individual was withdrawn.
k. Data from electronic datasets and NDA volumes 2.14, 2.16, 2.17 and 2.29.
l. Subject in the INO-01/ -02 could be classified as a treatment failure even if their treatment gas was decreased rather than stopped.
m. Cardio-pulmonary instability was not a reason used for treatment failure in the NINOS summary data sheets.

8.1.3.2 Adverse Events Associated with Dropout

For reasons discussed in previously, the NINOS and INOSG trials collected little information regarding specific adverse events, including adverse events associated with withdrawal from the trial. Below is a listing of the adverse events associated with dropout in the INO-01/ -02 and INO-03 trials, followed by a narrative for each case. This will be followed by a brief discussion of the selected adverse events which were reported in association with dropout in the NINOS and INOSG trials.

8.1.3.2.1 Adverse events associated with dropout from the INO-01/ -02 trial

Table 8.1.3.2.1.1 Subjects from the INO-01/ -02 and INO-3 trials who dropped out due to adverse events[a].

Study Group	Subject #	Adverse Event	Outcome
Control			
	01-4009	Hypoxia	ECMO, Discharged without CLD[b]
I-NO 5 ppm			
	01-1004	Acute pulmonary decompensation Elevated LDH	ECMO, HFOV, Discharged without CLD
I-NO 20 ppm			
	No subjects		
I-NO 80 ppm			
	01-01005	Methemoglobin level >7%	No ECMO Discharged without CLD
	01-03003	Methemoglobin level >7%	No ECMO Discharged without CLD
	01-03016	Methemoglobin level >7%	No ECMO Discharged without CLD
	01-03029	Methemoglobin level >7%	No ECMO Discharged without CLD
	01-05005	Methemoglobin level >7%	No ECMO Discharged without CLD (some F/U data missing)
	01-06003	Methemoglobin level >7%	No ECMO Received HFOV Discharged without CLD (some F/U data missing)
	01-11004	Methemoglobin level >7%	No ECMO Discharged without CLD Had seizure disorder
	01-17004	Methemoglobin level >7%	No ECMO Discharged without CLD
	02-04004	Methemoglobin level >7%	No ECMO Discharged without CLD
	02-04006	Methemoglobin level >7%	Received ECMO Discharged without CLD
	02-07003	Methemoglobin level >7%	No ECMO Discharged without CLD
	02-13001	Elevated NO_2 level	No ECMO Discharged without CLD

a. Data comes from review of individual case report forms, NDA volumes 3.4-3.10 and from electronic data sets.
b. CLD chronic lung disease as defined in the INO-01/ -02 trial.

8.1.3.2.1 Patient narratives for dropouts or changes in therapy due to adverse events in the INO-01/ -02 trial

a. Control subjects

1. Patient 01-4009: 2.7 kg Hispanic female born to a mother with a history of crack and heroin addiction on methadone. The patient's Apgars were 9 and 9 and PPHN was diagnosed possibly secondary to sepsis. Her initial LDH was 3957 and SGOT 102 (before I-NO). She showed no response to the control gas (PaO_2 52 to 38 after 30 minutes), and the subject was declared a treatment failure and withdrawn from study gas for severe hypoxia. She received ECMO with improvement in oxygenation, and was discharged. Her LFT abnormalities resolved.

8.1.3.2.1 Patient narratives for dropouts or changes in tx due to adverse events in the INO-01/ -02 trial (cont)

b. I-NO (5 ppm) subjects

1. Patient 01-1004: a 3.6 kg white male, born to a women who received treatment for a UTI with Bactrim during the pregnancy. The patient's Apgars were 8 and 9, and meconium staining was noted. PPHN from meconium aspiration developed and he was started on study gas (I-NO 20 ppm), with an acute increase in PaO_2 (41 to 82), followed by a gradual decline in oxygenation over several hours, despite continued I-NO. After 44 hours of therapy the subject suffered an acute decompensation (PaO_2 39), and was emergently weaned by the investigator from the I-NO and placed on ECMO and HFOV. Infant ultimately recovered and went home without O_2 therapy.

c. I-NO (20 ppm) subjects

No subjects

d. I-NO (80 ppm) subjects

1. Subject 01-1005: 2.7 kg white female, born to a woman with oligohydramnios, with decreased fetal movement prior to delivery by emergency Cesarean section. Apgars were 3 and 6. PPHN was diagnosed with meconium present below the cords. Placed on I-NO, 80 ppm, with an acute increase in PaO_2 (47 to 82). After 36 hours of I-NO, her methemoglobin levels were >7%, and she was weaned off I-NO for this reason. After weaning there was no evidence of decreased PaO_2. She recovered without ECMO and was discharged home.

2. Subject 01-3003: 3.1 kg white female, born to a woman with a history of metamphetamine use and hypertension, had Apgars of 6 and 8. PPHN was diagnosed with meconium aspiration and subject was started on I-NO 80 ppm. SGOT, Alkaline Phosphatase and LDH were elevated pre-I-NO, which returned to normal after treatment. She had an acute response to I-NO (PaO_2 93 to 202), but was weaned after 16 hours after a single methemoglobin >7% (7.3%). After weaning there was no evidence of decreased PaO_2. She survived without ECMO and was discharged home.

3. Subject 01-3016: 3.5 kg Hispanic male, born by cesarean section after prolonged bradycardia. Apgars were 0 and 3, and PPHN developed due to possible sepsis. He was started on I-NO, 80 ppm, with an acute response (PaO_2 55 to 292). After 4 hours, subject was weaned for methemoglobin >7% (7.2 and 7.9%). Following discontinuation, PaO_2 declined from 285 to 156 (7 hrs later) and 105 (17 hours later). Subject was weaned the next day without ECMO, and was discharged from the hospital.

4. Subject 01-3029: 3.9 kg Hispanic male, born vaginally with meconium staining. Apgars were 2 and 8, and PPHN developed. LDH and SGOT were elevated on admission (907 and 134 IU/L respectively). These resolved slowly during the hospitalization. Subject was started on I-NO, 80 ppm, with no response (PaO_2 52 to 59 after 30 minutes). Due to elevated methemoglobin of (11.9 after 5 hours), subject was discontinued from I-NO. Oxygenation acutely declined (PaO_2 95 to 48), and the subjects clinical state deteriorated. He was restarted on I-NO, 20 ppm with slow improvement in PaO_2. When weaning was attempted approximately 48 hours later, he again had a decline in PaO_2 (to 48 mmHg), and I-NO was re-instituted. Weaning was successful with the next attempt and the subject was discharged without receiving ECMO.

Comment: this infant had an acute decline in his PaO_2 on two occasions after weaning from I-NO.

5. Subject 01-5005: 4.0 kg white male, developed PPHN after an uncomplicated delivery. He received I-NO, 80 ppm, with an acute increase in PaO2 (62 to 134 after 30 minutes) which persisted for 12 hours. After 12 hours the study gas had to be decreased due to methemoglobin >7% (7.6%). His methemoglobin levels fell, and the subject was gradually weaned after 6 days. He did not receive ECMO, and was discharged home.

6. Subject 01-6003: 3.2 kg black male, born without complications, developed PPHN, possibly secondary to pneumonia. Apgars were 9 and 9. His LFTs were elevated on entry into study and remained high. This was attributed to the hypoxia by the investigator. He was placed on I-NO, 80 ppm, without significant response (PaO_2 50 to 56 after 30 minutes), and without response for the first 4 hours. Elevated methemoglobin levels developed immediately (7.5% after 4 hours), and I-NO was reduced and then discontinued. PaO_2 fell from 73 to 51, 30 minutes after the reduction. Subject received HFOV rescue, but no ECMO, and was discharged home.

7. Subject 01-11004: 3.4 kg Native American female, born with abruptio placenta and after late deceleration's by cesarean section. Her Apgars were 1 and 5, in association with seizures. PPHN was felt secondary to possible sepsis. She had markedly elevated LFTs on admission (LDH 3429, SGOT 503). These persisted through the I-NO treatment period. She was started on I-NO, 80 ppm, with acute improvement in her PaO_2 (53 to 265 after 30 minutes). Treatment gas was weaned and then stopped due to elevated methemoglobin levels (7.6 after 8 hours). She recovered without ECMO.

8.1.3.2.1 Patient narratives for dropouts or changes in tx due to adverse events in the INO-01/ -02 trial (cont)

8. Subject 01-17004: 3.5 kg white female born by cesarean section with meconium staining. She received I-NO, 80 ppm, with an acute increase in PaO_2 (74 to 144 after 30 minutes) which persisted through the first 8 hours. The I-NO had to be decreased to 40 and then 20 ppm for increased methemoglobin levels (9.3% after 7.5 hours). The levels fell as the I-NO was reduced. After weaning successfully after 149 hours of I-NO, she recovered and was discharged without receiving ECMO.

9. Subject 02-4004: 3.0 kg black female born vaginally to a mother with a history of cocaine and crack abuse. She developed PPHN and bilateral pneumothoraces, and was started on I-NO, 80 ppm, without significant acute or sustained increase in PaO_2 (44 at baseline to 58 mmHg after 30 minutes). After 4 hours the I-NO had to be weaned then discontinued for elevated methemoglobin (8.6% after 4 hours). She recovered without ECMO and was discharged home. Her baseline hypoxia did not worsen acutely after weaning off I-NO.

10. Subject 02-4006: 2.7 kg black male was born by cesarean section with meconium stained amniotic fluid. His LFTs were markedly abnormal at baseline (LDH 3841, SGOT 269) and he had renal insufficiency (creatinine 1.1). He was started on I-NO, 80 ppm, with no acute improvement in PaO_2 (79 at baseline to 51 after 30 minutes). His gas did improve by 3 hours on I-NO (PaO_2 161). However, because of elevated methemoglobin (10.8% after 2.5 hours), he was weaned from study gas. He slowly improved clinically, the methemoglobin level fell, and he tolerated the weaning without acute hypoxia after 143.5 hours of I-NO at < 80 ppm.

11. Subject 02-7003: 4.1 kg black male born by Cesarean section after prolonged bradycardia, with perinatal asphyxia and meconium staining. He developed markedly elevated LFTs before starting I-NO (LDH 3055, SGOT 341) as well as renal failure (creatinine 1.6 before starting study gas). The LFTs improved slightly during the I-NO administration, while the renal failure worsened (creatinine 2.0 after 24 hours). For his PPHN he was started on I-NO, 80 ppm, with an acute increase in PaO_2 (69 at baseline to 173 after 30 minutes). He developed elevated methemoglobin (7.8% after 8 hours) and the study gas was weaned off. He ultimately improved with ECMO and was discharged to home.

12. Subject 02-13001: 2.9 kg white male was born vaginally to a woman with a history of asthma. He developed PPHN secondary to a suspected aspiration pneumonia. He was begun on I-NO, 80 ppm, without acute change in PaO_2 (50 at baseline to 49 after 30 minutes). The initial NO_2 level was reported as 5.8, and the subject was tapered off the I-NO. It was later determined that the NO_2 monitor was 'coiled', and that once it was fixed, the value for the subject's NO2 was within normal limits. However, he did not respond to I-NO, and the gas was discontinued. He recovered without the use of ECMO and was discharged.

13. Subject 01-4005: a case report form is not available for this 2.9 kg Hispanic male with idiopathic PPHN. He had a methemoglobin level of 7.3 after 8 hours, along with an NO_2 level of 2.5, and he was tapered of I-NO by 20 hours. He did not receive ECMO and was discharged home.

8.1.4 Other Significant Adverse Events

No information is available regarding adverse events which led to changes in therapy, with the exception of elevated methemoglobin and NO_2 levels.

8.1.5 Adverse Event Incidence

8.1.5.1 Approach to Eliciting Adverse Events in the Development Program

8.1.5.1.1 Adverse event collection in the four trials

As discussed at the beginning of the safety review, the four trials (NINOS, INO-01/ -02 and /-03, and INOSG) each took a very different approach to the collection of adverse events. The table below summarizes the types of safety information collected in each trial.

Table 8.1.5.1 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.

8.5.1a Adverse event collection in the INO-01/ -02 and INO-03 trials

For the INO-01/ -02 and INO-03 trials, an adverse event was defined as 'Treatment Emergence Signs and Symptoms (TESS). These are events that are not seen at baseline or, if present at baseline, have worsened in severity.' (Ohmeda Adverse Event Form). Investigators were also instructed to contact Ohmeda immediately with any serious adverse event.

'Adverse Event Report Forms' were filled out by the investigators. These were blank forms, with spaces for 'Adverse Event', history of same, time of onset, duration, severity, relationship to drug, actions taken and outcomes.

The INO-01/ -02 trial also had a sheet which was filled out prior to discharge by data managers. A series of check boxes were presented covering the presence or absence of specific adverse events during the hospitalization (see Table 8.1.5a.2.). In discussion with Dr. Rick Straube, the primary reviewer of the data for the sponsor, this data collection at the time of discharge was done by independent data monitors, rather than the individual investigators. This difference is important to keep in mind during the discussion of rates of occurrence of individual adverse events.

8.5.1a Adverse event collection in the NINOS trial

During the NINOS trial, a form was filled out for each infant summarizing the clinical course, therapies, and adverse events. No mechanism for recording all adverse events was in place. Rather, the form contains a series of check boxes to record the presence or absence of specific adverse events (see Table 8.1.5b.2). In discussion with Dr. Rick Straube, the primary reviewer of the data for the sponsor, the data collection at the time of discharge was done by independent data monitors, rather than the individual investigators.

8.5.1a Adverse event collection in the INOSG trial

During the INOSG trial, a form was filled out for each infant summarizing the inclusion and exclusion criteria, and the initial clinical presentation of the infant. No mechanism for recording all adverse events was in place. A follow-up questionnaire was apparently filled out prior to discharge, which gives check boxes for 8 separate adverse events (O_2 therapy, diuretic therapy, anti-convulsant therapy, occurrence of seizures, patient weight, date of discharge, occurrence of hospitalizations for pneumonia after discharge, and follow-up).

A summary of specific adverse events followed in each trial is below.

8.5.1a Adverse event collection in the INOSG trial

Table 8.1.5c.1 Specific adverse events collected during the NINOS, INOSG, INO-01/ -02 and INO-03 trials.

Adverse Event	NINOS	INOSG	INO-01/ -02	INO-03
All adverse events[b]	N	N	Y	Y
Routine serum chemistries & hematology	N	N	Y	Y
Adverse events specifically followed[c]				
ECMO	Y	Y	Y	Y
Death	Y	Y	Y	Y
Seizures	Y	N	Y	Y
Intracranial bleeding	Y	N	Y	Y
Peri-ventricular leukomalacia	Y	N	Y	Y
Brain Infarct	Y	N	Y	Y
Ventriculomegaly	N	N	Y	Y
Air leak syndrome	Y	Y	Y	Y
Chronic lung disease Bronchopulmonary dysplasia	Y	Y	Y	Y
Reactive airway disease	note a.			
Supplemental O_2 at 28 days	Y	Y	Y	Y
Diuretic therapy	N	Y	N	N
Pulmonary hemorrhage	Y	N	N	N
Prolonged bleeding elsewhere	Y	N	N	N
BAER hearing test	N	N	Y	N
NO_2 levels	Y	N	Y	Y
Methemoglobin levels	Y	Y	Y	Y

a. Reactive airway disease was subsumed under chronic lung disease in the NINOS trial.
b. For the selected adverse events the mechanisms used to collect them differed by study. These were discussed above.

8.1.5.1.2 Identifying Key Adverse Events

Several methods will be used to establish the key adverse events in the summary tables below. Key adverse event, in this usage, means an adverse event which will be discussed specifically in section 8.2 because it may be linked to the use of I-NO. First, any adverse event identified in the INO-01/ -02 trial occurring in >1% of the subjects in either the control or the I-NO populations will be tabulated, and the percentage compared. Any event which occurs ≥2x more frequently in the I-NO group from study INO-01/ -02 will be discussed in section 8.2 for it's relevance.

The critical tables from the sponsor that were used to identify adverse events were:

INO-01/ -02

1) Table 44, volume 2.17: listing of patients with specific adverse events.
2) Table 10.5.6-1, volume 2.50: percent of patients with adverse events.
3) Table 36, volume 2.17: reported adverse events.
4) Table 45, volume 2.17: listing of adverse events with death as an outcome.
5) Table 46, volume 2.17: listing of serious adverse events.
6) Table 47, volume 2.17: listing of serious adverse events by patient.
4) Electronic dataset 'Adverse'.

INO-03

1) Tables 25-27, volume 2.29: listing of patients with specific adverse events.
2) Tables 21-24, volume 2.29: reported adverse events.
3) Table 10.5.6-2, volume 2.50: percent of patients with adverse events.

NINOS

1) Appendix A, volume 2.15, toxicity and adverse events
2) Table T-9, volume 2.14, patients with elevated methemoglobin levels.

8.1.5.1.2 Identifying Key Adverse Events

In section 8.2, the adverse events will be scrutinized according to body system. The deaths and serious adverse events will be integrated into the data on less severe, related, adverse events. For instance, the subjects who were withdrawn from a study due to methemoglobinemia and elevated NO_2 will be scrutinized for evidence of chronic pulmonary damage, including asthma, and the pattern compared with the subjects in the INO-01/ -02/03 database who also developed asthma.

Next, the adverse events identified by each investigator were collated (see table 8.1.5.4.2 below). Any event which occurs ≥2x as often in the I-NO group as in the control group, or is life-threatening will be included.

Next, the selected adverse events which were identified prospectively as clinically relevant to the use of I-NO in the NINOS, INOSG, INO-01/ -02 and INO-03 trials will examined, and their relative incidence rates in the control and I-NO groups compared. These events are listed in table 8.1.5a.2 above. Data for these adverse events came from the individual study reports, as well as the relevant electronic datasets. Again, any event which occurs ≥2x as often in the I-NO group will be scrutinized in section 8.2 below.

Adverse events which are laboratory values will be examined in section 8.1.6 below. The methods used to detect these lab abnormalities are discussed in detail there.

Finally, because the potential toxicities of I-NO are difficult to predict, and the database is quite limited in size, the literature was reviewed with particular attention towards any unusual adverse events. This literature review will be reinforced by examining any data that exists in from the four trials in the NDA, as part of section 8.2 below. The small number of subjects which appear in the NDA database, sets boundaries on the detection of adverse events. A total of 128 subjects were exposed to I-NO in the INO-01/ -02 and INO-03 trials, and are the subjects for whom adverse event data, anticipated and unanticipated, was collected. This approximates the number of subjects necessary to detect one occurrence of an adverse event which occurs in 2% of the subjects with 95% confidence. This will limit the ability to perform meaningful analyses of dose-, age-, sex- and duration-dependence for low-frequency adverse events. The literature review helps to enlarge this safety database. While this literature search, by it's nature, cannot be quantitative, it is an important part of evaluating the potential uncommon and/or unanticipated adverse events due to I-NO.

8.1.5.2 Establishing Appropriateness of Adverse Event Categorization and Preferred Terms

In the trials that were submitted, only the INO-01/ -02 and INO-03 trials routinely used COSTART terms to describe the adverse events. As discussed above, the remaining trials were planned and carried out without systematic collection of all adverse events. In the INO-01/ -02 and INO-03 trials, the description of adverse events was appropriately detailed in almost all cases. Instances of use of multiple terms to describe a single clinical event will be discussed as they arise.

8.1.5.3 Selecting the Key Adverse Events Tables for Characterizing the Adverse Event Profile

8.1.5.4 Adverse events from the INO-01/ -02 and /-03 trials

The first table summarizes all reported adverse events according to the individual investigator's judgment as to the relationship of the event to the study gas (I-NO or N_2 control). The focus on the INO-01/ -02 and /-03 studies for the adverse events comes from the data available: the NINOS and INOSG collected data only on those safety parameters that were identified prospectively as clinically important. These defined safety parameters will be summarized separately below. There is a higher incidence of related adverse events in the 80 ppm I-NO group, largely driven by the increased incidence of methemoglobinemia. This is in contrast to the non-related adverse events, where the 80 ppm group and control have similar incidence rates.

Table 8.1.5.4.1 Total adverse event rates (AEs) from the INO-01/ -02 and /-03 trials.

	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Pooled I-NO
Total Subjects	41	45	44	39	128
Non-related AEs[b]	13/41 (32%)	17/45 (38%)	20/44 (45%)	13/39 (33%)	50/128 (39%)
Related AEs[c]	0/41 (0)%	3/45 (7%)	1/44 (2%)	17/39 (44%)	21/128 (16%)
All AEs[a]	14/41 (32%)	19/45 (42%)	21/44 (47%)	23/39 (59%)	70/128 (54%)

a. Subjects who had more than one AE are counted once by severity of AE.
b. Non-related AEs: judged to be 'unrelated' or 'remotely related' to study gas by the investigator.
c. Related AEs: judged to be 'possibly,' 'probably,' or 'definitely' related to study gas by the investigator.

The next table summarizes the occurrence of all adverse events, regardless of the inferred relationship to the drug, in the INO-01/ -02 and INO-03 trials.

8.1.5.4 Adverse events from the INO-01/ -02 and /-03 trials (cont)

Table 8.1.5.4.2 Reported adverse events from INO-01/ -02 and INO-03 trials with reported frequency >1% or having serious clinical implications, presented by frequency within each body system for subjects receiving control gas and each of the I-NO dosage groups[a].

Body System/ adverse experience	Control Group n=41	I-NO 5 ppm n=45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	Combined I-NO n=128
Any adverse event	13 (32%)	19 (42%)	21 (47%)	23 (59%)	63 (49%)
Body as a whole	4 (10%)	7 (16%)	4 (9%)	4 (10%)	15 (12%)
Hydrocephalus	1 (2%)	2 (4%)			2 (2%)
Hypertrophy	1 (2%)				
Sepsis	2 (5%)	1 (2%)	1 (2%)	1 (3%)	3 (3%)
Withdrawal syndrome[b]	1 (2%)	1 (2%)	1 (2%)		2 (2%)
Overdose[c]				2 (5%)	2 (2%)
Infection		2 (4%)	1 (2%)		3 (3%)
Injection site reaction		1 (2%)			1 (<1%)
Cellulitis			1 (2%)	1 (3%)	2 (2%)
Cardiovascular system	2 (5%)	5 (12%)	4 (9%)	7 (18%)	16 (12%)
Aortic Thrombosis	1 (2%)				
Arrhythmia		2 (5%)			2 (2%)
Atrial septal defect		1 (2%)			1 (<1%)
'Heart arrest'			1 (2%)	1 (3%)	2 (2%)
Hypertension			1 (2%)	1 (3%)	2 (2%)
Hemorrhage			1 (2%)	1 (3%)	2 (2%)
Cardiovascular disorder		1 (2%)			1 (<1%)
Hypotension		1 (2%)		1 (3%)	2 (<1%)
Bradycardia			1 (2%)	1 (3%)	2 (2%)
Phlebitis				1 (3%)	1 (<1%)
Vascular anomaly	1 (2%)			1 (3%)	1 (<1%)
Endocrine system	1 (2%)				
Adrenal insufficiency	1 (2%)				
Hemic & Lymphatic	2 (5%)	1 (2%)	5 (11%)	16 (41%)	22 (17%)
Anemia	1 (2%)		1 (2%)	1 (3%)	2 (2%)
Methemoglobinemia				13 (35%)	13 (11%)
Ecchymoses				1 (3%)	1 (<1%)
Hypovolemia		1 (2%)			1 (<1%)
Thrombocytopenia	1 (2%)		3 (7%)	1 (2%)	4 (3%)
Gastrointestinal system			1 (2%)	1 (3%)	2 (2%)
Gastrointestinal hemorrhage				1 (3%)	1 (<1%)
Gastrointestinal anomaly			1 (2%)		1 (<1%)

a. Data from NDA, volume 2.17, page 089808 to 092408, volume 2.29 page 353108 to 353308, and from individual case report forms.

b. Withdrawal syndrome refers to withdrawal from concomitant narcotic medications (in particular, fentanyl used during paralysis) and not to withdrawal from I-NO.

c. Overdose does not refer to an overdose of I-NO, and instead refers to concomitant medications.

8.1.5.4 Adverse events from the INO-01/ -02 and /-03 trials

Table 8.1.5.4.2 (cont) Reported adverse events from INO-01/ -02 and INO-03 trials with reported frequency >1% or having serious clinical implications, presented by frequency within each body system for subjects receiving control gas and each of the I-NO dosage groups[a].

Body System/ adverse experience	Control Group n=41	I-NO 5 ppm n=45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	Combined I-NO n=128
Metabolic & Nutritional	4 (10%)	5 (11%)	7 (16%)	3 (8%)	15 (12%)
Bilirubinemia	2 (5%)	4 (9%)	3 (6%)	1 (3%)	8 (6%)
Hypokalemia	1 (2%)		1 (2%)		
Hypoglycemia				1 (3%)	1 (<1%)
Hyperglycemia			1 (2%)		1 (<1%)
Hypocalcemia		1 (2%)	1 (2%)		2 (2%)
Calcium disorder			1 (2%)		1 (<1%)
Hyponatremia	1 (2%)			1 (3%)	1 (<1%)
Nervous system	4 (10%)	2 (4%)	9 (20%)	5 (13%)	16 (12%)
Cerebral infarct	1 (2%)		1 (2%)		1 (<1%)
Cerebrovascular disorder	1 (2%)			1 (3%)	1 (<1%)
Encephalopathy			1 (2%)	1 (3%)	2 (2%)
Subdural hematoma			1 (2%)		1 (<1%)
Paralysis				1 (3%)	1 (<1%)
Convulsion	2 (5%)	2 (4%)	5 (11%)	2 (5%)	9 (7%)
Abnormal electroencephalogram			1 (2%)		
Musculoskeletal system				1 (3%)	1 (<1%)
Pathological fracture				1 (3%)	1 (<1%)
Respiratory system	7 (17%)	10 (22%)	11 (25%)	9 (23%)	30 (23%)
Asphyxia	1 (2%)		1 (2%)	1 (3%)	2 (2%)
Chondromalacia	1 (2%)	1 (2%)			1 (<1%)
Hypoxia	1 (2%)	1 (2%)			1 (<1%)
Lung disorder	2 (5%)	2 (5%)	3 (3%)	2 (3%)	7 (6%)
Pneumonia	1 (2%)				
Asthma		2 (2%)	2 (3%)	1 (2%)	5 (4%)
Pneumothorax	1 (2%)	4 (10%)	3 (8%)	3 (8%)	10 (8%)
Emphysema			1 (2%)		1 (<1%)
Lung edema			1 (2%)		1 (<1%)
Pleural effusion				1 (3%)	1 (<1%)
Respiratory congenital anomaly				1 (3%)	1 (<1%)
Stridor	1 (2%)				
Genitourinary system	1 (2%)	3 (7%)	3 (7%)		6 (6%)
Kidney failure	1 (2%)		2 (3%)		2 (2%)
Kidney abscess		2 (5%)			2 (2%)
Urogenital anomaly			1 (2%)		
Acute tubular necrosis		1 (2%)			1 (2%)
Special senses				1 (3%)	1 (<1%)
Deafness				1 (3%)	1 (<1%)

a. Data from NDA, volume 2.17, page 089808 to 092408, volume 2.29 page 353108 to 353308, and from individual case report forms.

8.1.5.6 Selected Adverse Events Collected from All Trials.

The second approach to the safety database is to examine occurrence of the specified adverse events from all of the trials. Data for these specific adverse events was collected prospectively in most of the trials. Each of them was felt by the investigators to be of critical importance in gauging safety in a neonatal population. For some safety endpoints (reactive airway disease, sensorineural hearing loss), only one trial collected data. Additionally, the NINOS and INOSG trials enrolled a 'sicker' population than the INO-01/ -02, which limits the interpretation of the pooled data for all of these endpoints. These safety endpoints will be further discussed in the appropriate body system in section 8.2.

8.1.5.6a Incidence of deaths from all trials

Table 8.1.5.6a.1 Reported deaths from NINOS, INOSG, INO-01/ -02 and INO-03[a].

Trial	Control	I-NO	p value[b]
NINOS	20/121 (16.5%)	16/114 (14%)	0.6
INOSG	3/28 (10/7%)	2/30 (6.7%)	0.7
INO-01/ -02	2/41 (4.9%)	10/113 (8.8%)	0.42
INO-03	No control	0/14	NA
Total	25/190 (13.2%)	28/271 (10.3%)	0.43

a. Data from NDA volume 2.50, page 339310 and electronic datasets.
b. p value for all long-term comparisons using Cochran-Mantel Haenszel chi-squared test vs. control for all subjects with data.

8.1.5.6b Incidence of intracranial hemorrhages from all trials

Table 8.1.5.6b.1 Reported intracranial hemorrhages from NINOS, INOSG, INO-01/ -02 and INO-03[a].

Trial	Control	I-NO	p value
NINOS	19/121 (15.7%)	18/114 (15.9%)	0.87
INOSG	Not available	Not available	NA
INO-01/ -02	3/41 (7.3%)	6/114 (5.3%)	0.44
INO-03	No control	1/8 (12.5%)	NA
Total	22/162 (13.6%)	25/236 (10.6%)	0.45

a. From NDA volumes 2.14, 2.16 and 2.17 and electronic datasets

8.1.5.6c Incidence of seizures from all trials

Table 8.1.5.6c.1 Reported seizures from NINOS, INOSG, INO-01/ -02 and INO-03[a].

Trial	Control	I-NO	p value
NINOS	24/121 (19.8%)	16/114 (14%)	0.31
INOSG	Not available	Not available	NA
INO-01/ -02	7/41 (17%)	22/112 (19.6%)	0.9
INO-03	No control	3/13 (23%)	NA
Total	31/162 (19.1%)	41/239 (17.2%)	0.71

a. From NDA volumes 2.14, 2.16 and 2.17 and electronic datasets

8.1.5.6d Incidence of bronchopulmonary dysplasia from all trials

Table 8.1.5.6d.1 Reported bronchopulmonary dysplasia from NINOS, INOSG, INO-01/ -02 and INO-03[a,c].

Trial	Control	I-NO	p value
NINOS	14/101 (15%)	16/100 (16%)	0.71
INOSG[b]	4/28(14%)	1/30 (3%)	0.18
INO-01/ -02	5/40 (13%)	13/103 (12%)	1.0
INO-03	No control	3/14 (21%)	NA
Total	23/169 (13.6%)	33/249 (13.2%)	1.0

a. Bronchopulmonary dysplasia defined as requiring bronchodilators or O_2 on discharge with abnormal chest x-ray.
b. Patient data missing for several subjects. Data represents those subjects receiving O_2 at day 28.
c. From NDA volumes 2.14, 2.16 and 2.17 and electronic datasets.

8.1.5.6e Incidence of dependence on supplementary O_2 at time of discharge

Table 8.1.5.6e.1 Incidence of use of supplemental O_2 at time of discharge in the NINOS, INO-01/ -02 and /-03 and INOSG trials[a].

Trial	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
NINOS	15/100 (15%)		14/98 (14%)		
INO-01/ -02 and /-03	6/41 (15%)	9/45 (20%)	4/43 (9%)	7/39 (18%)	20/127 (16%)
INOSG[b]	4/21 (19%)			1/27 (4%)	

a. Data from NDA, volume 2.26 appendix 16.2.2.21, and 2.31 data listing 16.5. Data shown as % of all subjects with data. INOSG data from NDA volume 2.16, Appendix 16.2.7.
b. p value for control vs. I-NO 0.19.

8.1.5.6f Incidence of initiation of ECMO from all trials

Table 8.1.5.6f.1 Rate of the initiation of ECMO in the NINOS, INOSG, and INO-01/ -02 trials[b].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value
NINOS[a]	66/121 (54.5)				44/114 (38.5%)	0.014
NINOS[c]	62/112 (55%)		0.067[c]		48/118 (41%)	0.067[c]
INOSG[a]	20/28 (71%)			12/30 (40%)		0.0198
INO-01/ -02[a]	14/41 (34%)	10/41 (24%)	9/36 (25%)	6/37 (16%)	25/114 (22%)	0.34

a. Based on ITT population, p value calculated using unadjusted chi-square.
b. Data from individual study reports, NDA volumes 2.14, 2.16 and 2.17, and electronic datasets.
c. Based on 'gas received' population. p value calculated using Cochran-Mantel-Haenszel adjusted chi-square test.

8.1.5.6g Incidence of long-term adverse events from the INO-01/ -02 trial

In the INO-01/ -02 trial, surviving infants were to have a follow-up assessment after 1 year. Several safety endpoints were to be assessed, including: vital status; medical history (including hospitalizations); neurologic and physical development; and audiology. The first table shows the extent of follow-up for the various tests. It's important to note that that the incomplete nature of the follow-up may introduce bias. Since infants with poor outcomes tend to be hospitalized more often, their records are frequently more complete.

Vital Status

Table 8.1.5.6g.1 Vital status at 1 year of age for infants for infants with known follow-up in INO-01/ -02.

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	41	41	36	37	114
Alive	36 (95%)	35 (95%)	29 (85%)	31 (91%)	95 (90.5%)
Dead	2 (5.3%)	2 (5.4%)	5 (14.7%)	3 (8.8%)	10 (9.5%)
p value[a]		0.978	0.180	0.556	0.419

a. p value calculated using Cochran Mantel Haenzsel chi-squared test.

The next tables show the incidence of the individual adverse events.

Mental development

Table 8.1.5.6g.2 Mental development at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
Accelerated development	2 (6%)	2 (5%)	1 (3%)	2 (6%)	5 (5%)
Normal development	21 (58%)	20 (57%)	17 (59%)	17 (55%)	54 (57%)
Mildly delayed development	7 (19%)	6 (17%)	2 (7%)	5 (16%)	13 (14%)
Significantly delayed development	2 (6%)	0 (0%)	4 (14%)	2 (6%)	6 (6%)
Missing	3 (8%)	5 (14%)	2 (7%)	3 (10%)	10 (10%)

a. Subjects were tested using the Bayley Scales of Infant Development and the standardized Mental Development Index (MDI) was calculated.

MDI ≥115	Accelerated development
85_MDI<115	Normal development
70_MDI<85	Mildly delayed development
MDI<70	Significantly delayed development

8.1.5.6g Incidence of long-term adverse events from the INO-01/ -02 trial (cont)

Psychomotor development

Table 8.1.5.6g.3 Psychomotor development at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
Accelerated development	2 (6%)	4 (11%)	1 (3%)	2 (6%)	7 (7%)
Normal development	24 (67%)	20 (57%)	16 (55%)	21 (67%)	57 (60%)
Mildly delayed development	4 (11%)	2 (6%)	3 (10%)	1 (3%)	6 (6%)
Moderately delayed development	0 (0%)	3 (8.6%)	2 (6.9%)	1 (3.2%)	6 (6%)
Significantly delayed development	2 (6%)	1 (3%)	4 (14%)	3 (10%)	8 (8%)
Missing	4 (11%)	5 (14%)	3 (10%)	3 (10%)	11 (12%)

a. Subjects were tested using the Bayley Scales of Infant Development and the standardized Psychomotor Development Index (PDI) was calculated.

PDI ≥115	Accelerated development
85≤PDI<115	Normal development
70≤PDI<85	Mildly delayed development
50≤PDI<70	Moderately delayed development
PDI<50	Significantly delayed development

Audiology testing

Table 8.1.5.6g.4 Results of audiology testing at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
None	22 (61%)	22 (63%)	19 (66%)	18 (58%)	59 (62%)
Mild	7 (19%)	2 (6%)	4 (14%)	3 (10%)	9 (10%)
Major	0 (0%)	1 (3%)	0 (0%)	1 (3%)	2 (2%)
Missing	7 (19%)	10 (29%)	6 (21%)	9 (29%)	25 (26%)

a. Subjects were tested using pure-tone audiologic testing at 0.5, 1, and 2 kHz. Abnormalities were categorized according to loss of audible threshold.

Threshold ≤25 dB	None
>25 to <50 dB	Mild
≥50 dB	Major

Neurologic testing

Table 8.1.5.6g.5 Results of neurologic testing at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
None	28 (78%)	30 (86%)	20 (69%)	22 (71%)	72 (76%)
Mild	3 (8%)	1 (3%)	2 (7%)	1 (3%)	4 (4%)
Major	4 (11%)	3 (9%)	5 (17%)	5 (16%)	13 (14%)
Missing	1 (3%)	1 (3%)	2 (7%)	3 (10%)	6 (6%)

a. Examining physicians were asked to characterized neurologic abnormalities as none, mild or major.

Incidence of Cerebral Palsy

Table 8.1.5.6g.6Incidence of cerebral palsy at 1 year of age for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
None	33 (92%)	34 (97%)	24(83%)	25 (81%)	83 (87%)
Present	2 (6%)	0 (0%)	4 (14%)	3 (10%)	7 (7%)
Missing	1 (3%)	1 (3%)	1 (3%)	3 (10%)	5 (5%)

a. Examining physicians were asked to record whether the subject had cerebral palsy or not.

8.1.5.6g Incidence of long-term adverse events from the INO-01/ -02 trial (cont)

Incidence of respiratory abnormalities

At the 1 year follow-up visit, the family members were asked if the infant had any of the following pulmonary problems: home oxygen therapy; asthma; bronchiolitis; bronchitis; pneumonia; upper respiratory infection with severe cough; and smoking in household. Those subjects for whom the answer was 'yes' are tabulated below.

Table 8.1.5.6g.7 (from table 6.0.3.13.3.8) Respiratory system abnormalities at 1 year of age for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
Home O_2 therapy	0/36 (0%)	8/35 (23%)	1/29 (3%)	4/31 (13%)	13/95 (14%)
Asthma	5/36 (14%)	6/35 (17%)	3/29 (10%)	3/31 (10%)	12/95 (13%)
Bronchiolitis	5/36 (14%)	7/35 (20%)	4/29 (14%)	1/31 (3%)	12/95 (13%)
Bronchitis	2/36 (6%)	3/35 (9%)	3/29 (10%)	2/31 (6%)	8/95 (8%)
Pneumonia	2/36 (6%)	2/35 (6%)	2/29 (7%)	3/31 (10%)	7/95 (7%)
Upper respiratory tract infection	10/36 (28%)	2/35 (6%)	2/29 (7%)	4/35 (11%)	8/95 (8%)

a. From individual case report forms for long-term follow-up, Volumes 7.1 through 7.12.

The individual subjects identified as requiring supplemental O_2 during the 1 year following discharge are listed below in bold letters, along with their treatment group, duration of exposure to I-NO, maximum recorded levels of NO_2 and methemoglobin, whether they received HFOV/HFJV or surfactant, and the number of months they required O_2 after discharge. The infants with missing follow-up records are shown as 'Unknown.' Note that the two individuals with the highest methemoglobin levels, subjects 02-13003 and 01-03029, do not have follow-up data available. The two individuals with the highest NO_2 levels, 01-03029 and 02-07005, are likewise missing follow-up data.

There is no apparent relationship between the use of jet or oscillatory ventilation, or the administration of surfactant, and the need for long-term O_2.

The infants in normal face required supplemental O_2 at the time of discharge, but did not use it during the next 1 year. One infant, 01-07002 (shown bold and underlined) did not need supplemental O_2 at time of discharge, but required it during the one-year following discharge. Overall, 12 of the 17 (70%) infants who needed O_2 at time of discharge in the I-NO group required it during the one year following discharge.

The average length of time on supplemental O_2 after discharge for the infants who required it in the I-NO group was 3.6 months.

8.1.5.6g Incidence of long-term adverse events from the INO-01/ -02 trial (cont)

Table 8.1.5.6g.8 Subject in the INO-01/ -02 trial who used supplemental O_2 during the 1 year after discharge[a].

Subject #	Duration of study gas	Peak NO_2 level	Peak MetHgb level	Received HFOV/HFJV?	Received Surfactant?	Months on Supplemental O_2
Control group						
01-03006	7	0.1	0.6	HFOV	N	Unknown
01-03019	16	0.2	0.6	HFOV	N	Unknown
01-07001	2	0.4	0.4	HFJV	Y	0
01-09001	152	0.8	0.4	HFOV	N	0
02-04001	200	0.1	0.5	HFJV	Y	Unknown
02-12002	3	0.0	0.7	HFJV	Y	Unknown
I-NO 5 ppm						
01-03017	18	0	0.5	HFOV	N	3
01-04007	13	0.1	0.3	HFOV	N	4
01-06002	51	2	5.6	HFOV	Y	11
01-07008	82	2	0.7	None	N	3
01-17005	155	0.2	1.1	None	N	4
02-12003	41	0.7	0.5	None	N	2
02-13003	8	1.9	6.2	HFJV	N	Unknown
02-14001	140	0	1.4	None	N	4
02-14002	98	0.1	1.6	Both	N	0
01-07002	12	0.6	1.1	None	N	1
I-NO 20 ppm						
01-04006	21	0.3	0.9	HFOV	Y	0
01-11015	144	0.1	0.7	Both	Y	0 (died)
02-14007	3	0	0.4	Both	Y	2
I-NO 80 ppm						
01-02003	9	2	3	HFOV	Y	0
01-03029	123	3	11.9	None	N	Unknown
01-11006	52	2	3.8	HFOV	N	5
02-12001	25	0.8	3.6	None	N	4
02-07005	14	3	2.9	None	N	Unknown
02-14003	33	2	7.3	HFOV	Y	2
02-14005	18	1.6	3.6	HFOV	Y	3

a. Data from electronic datasets and 1 year follow-up individual case report forms.

8.1.5.6 Adverse event analyses by race, sex and age

This section will focus on the incidence of death and/ or ECMO in the database, as these are the adverse events which occurred with the highest frequency.

8.1.5.6.1 Demographics of deaths from all trials, analyzed by race and sex

The first table shows the deaths which occurred during the 4 trials prior to discharge, broken down by the race and sex of each infant. The data is expressed as a percentage of the total number of subjects that entered the trials.

Table 8.1.5.6.1.1 Deaths in the NINOS, INOSG and INO-01/ -02 and /-03 trials, broken down by race and sex of the individuals.

Demographic Group	Number of control subjects (% of all control subjects deaths, n=25)	Number of I-NO subjects (% of all I-NO subject deaths, n=28)
Sex		
Male	17 (61%)	14 (50%)
Female	8 (39%)	14 (50%)
Race		
White	16 (64%)	21 (75%)
Black	2 (8%)	3 (11%)
Hispanic	2 (8%)	3 (11%)
Asian	2 (8%)	1 (3%)
Other and unknown	3 (12%)	0 (0%)

8.1.5.6.1 Demographics of deaths from all trials, analyzed by race and sex (cont)

These demographics can be compared with the combined demographics from the NINOS, INOSG and INO-01/ -02 and /-03 trials. A higher percentage of the deaths occurred in the white subjects relative to their % in the overall population. Similarly, a lower fraction of the deaths occurred in the black and Hispanic populations relative to the % in the overall population.

Table 8.1.5.6.1.1 Demographics of the I-NO NDA database[a].

Demographic Parameter	Control	I-NO
Total	190	271
Sex		
Male	121 (63%)	146 (54%)
Female	67 (37%)	123 (46%)
Race		
White	105 (55%)	148 (55%)
Black	34 (18%)	56 (21%)
Hispanic	31 (16%)	41 (15%)
Asian	2 (1%)	5 (3%)
Other	13 (7%)	18 (7%)
Missing	5 (3%)	4 (1%)

a. Data from electronic datasets and NDA volume 2.29.

8.1.5.6.2 Demographics of ECMO from all trials

The next table shows the subjects who received ECMO during the 4 trials prior to discharge, broken down by the race and sex of each infant. The data is expressed as a percentage of the total number of a given population which entered the trials. No large differences between the make-up of the general population and the infants who received ECMO is noted.

Table 8.1.5.6.2.1 Subjects who received ECMO in the NINOS, INOSG and INO-01/ -02 and /-03 trials, broken down by race and sex of the individuals.

Demographic Group	Number of control subjects (% of all control subjects with ECMO, n=100)	Number of I-NO subjects (% of all I-NO subject with ECMO, n=84)
Sex		
Male	66 (65%)	48 (57%)
Female	34 (34%)	36 (45%)
Race		
White	53 (53%)	45 (54%)
Black	20 (20%)	18 (21%)
Hispanic	17 (17%)	14 (17%)
American Indian	2 (2%)	0 (0%)
Asian	4 (4%)	4 (5%)
Other and missing	5 (5%)	3 (4%)

a. Data from electronic datasets and NDA volume 2.29.

8.1.5.8 Common and Drug-Related Adverse Events

Identifying drug-related adverse events is, by necessity, non-quantitative in this NDA. To identify adverse events which might be linked to I-NO use, those events which occurred at an incidence of at least 5% for patients assigned to I-NO and for which the NDA drug incidence is at least twice the placebo incidence were collated. Secondary data sources were also used to suggest plausible events linked to I-NO (e.g., increased bleeding following I-NO use). The results of these investigations are included in section 8.2 below.

8.1.6 Laboratory Findings

8.1.6.1 Extent of Laboratory Testing in the Development Program

The third category of adverse events which occurred during the trials relate to abnormalities in laboratory measurements. Chemistries, hematology, and urinalyses are performed on all severely ill subjects, such as were enrolled in the four trials. Unfortunately, the database submitted as part of the NDA does not include most of this information. The NINOS and INOSG trials, conducted under academic investigator INDs, did not collect or submit routine chemistries, electrolytes, hematology or urinalyses, and no data is available. The INO-01/ -02 and INO-03 trials collected two sets of chemistries and hematology per protocol: one set at baseline and another set no more than 12 hours after discontinuation of treatment gas. No urinalyses, serum electrolytes (Na^+, K^+, Cl, HCO_3), or tests of bleeding parameters were submitted as part of the INO-01/ -02 study results, including the case report forms. This was in agreement with the initial IND protocol, under which INO-01/ -02 and INO-03 were performed.

Table 8.1.6.1.1 The number of subjects with laboratory values submitted as part of the NDA from trials INO-01/ -02 and INO-03[a].

Laboratory	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Baseline and follow-up chemistries	38	45	41	37	123
Baseline chemistries only	3	0	3	1	4
Baseline and follow-up hematology	38	41	32	38	111
Baseline hematologies only	3	0	2	0	2
Serum electrolytes (Na^+, K^+, Cl, HCO_3)	0	0	0	0	0
Urinalyses	0	0	0	0	0

a. Data from NDA volume 2.25.

Table 8.1.6.1.2 The number of subjects with laboratory values submitted as part of the NDA from the NINOS, INOSG, INO-01/ -02 and INO-01/ -02-03 trials[a,b].

Laboratory	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Methemoglobin levels	137	45	66	81	192
NO_2 levels	145	45	67	72	184

a Data from NDA volumes 2.16, 2.25, 2.26, 2.31, submitted electronic datasets, and case report forms for individual subjects.

b. Numbers refer to subjects with a minimum of baseline data available. In the NINOS trial, subjects who were exposed to 80 ppm I-NO are in the 80 ppm group, even though they had previously received 20 ppm as well per protocol.

8.1.6.2 Standard Analyses and Explorations of Laboratory Data

The following data were used to detect adverse events.

First, as part of the INO-01/ -02 and INO-03 trials, all observed adverse events were collected and categorized. This database will serve as the only source for controlled data on the occurrence of all adverse events, whether expected or not. The sources of the lab values for the INO-01/ -02 and INO-03 trials are the electronic datasets as well as the submitted NDA. Within the NDA, several sources of data exist. First, the summary tables provided by the sponsor for the laboratory adverse events identified by the investigators were examined, and identified lab abnormalities compared between the control and I-NO groups. These tables include the following critical tables provided by the sponsor:

INO-01/ -02

1) Appendix 16.2.2.12, volume 2.25: listing of clinical laboratory for individual patients- chemistries.

2) Appendix 16.2.2.13: listing of clinical laboratory for individual patients- hematology.

3) Table T-30, volume 2.18: new abnormal lab values within 12 hours of discontinuation of study gas.

4) Table T-41, volume 2.17: listing of patients with specific adverse events.

5) Tables T-21 to T-24, volume 2.18: change in clinical chemistries from screening to <12 hours after discontinuation of study gas.

6) Tables T-25 to T-29, volume 2.18: change in hematology values from screening to <12 hours after discontinuation of study gas.

7) Electronic dataset 'Lab' and ' Adverse'.

8.1.6.2 Standard Analyses and Explorations of Laboratory Data (cont)

INO-03

1) Data listing 13.1, volume 2.31: clinical laboratory listing for individual patients--chemistries.

2) Data listing 13.2, volume 2.31: clinical laboratory listing for individual patients--hematology.

3) Table 26, volume 2.29: listing of patients with specific adverse events.

4) Table 36, volume 2.29: new abnormal laboratory values within 12 hours of discontinuation of study gas.

5) Tables 29 to 32, volume 2.29: change in clinical chemistries from screening to <12 hours after discontinuation of study gas.

6) Tables 23 to 35, volume 2.29: change in hematology values from screening to <12 hours after discontinuation of study gas.

Individual case reports for the subjects who had an adverse event of interest were requested from the sponsor, and scrutinized where possible.

Separately, tables prepared by the sponsor detailing changes in routine lab values that occurred from baseline to 12 hours after discontinuation of study gas were used to identify adverse events of interest. This examination was done in two ways.

First, the mean values for all available data from single lab value at the baseline and post-treatment time points were calculated and compared for signs of a general effect on a lab parameter.

Second, the submitted tables of individual subject lab data were scrutinized for abnormal key lab values. Of the submitted labs, the key lab values chosen included the following: total bilirubin; SGOT; glucose; BUN; creatinine; calcium. These were deemed most critical in judging assessing adverse effects of I-NO. Individual lab values were looked at to detect 'outliers.' The incidence of these outliers was calculated and compared across the 4 treatment groups. Within these sets, subjects with markedly abnormal individual labs were identified (>2x upper limit or <0.5 lower limit). For these individuals, more detailed information, including follow-up labs, was requested of the sponsor

For all lab values except total bilirubin, normal was defined by the ranges printed in the individual datasets. For total bilirubin, the range of 'normal' was from 0.1 to 1.6 in some patients, and from 0.1 to 12 in others. To avoid confusion, total bilirubin values >12 were considered abnormal. Additionally, the sponsor was asked to examine the record for all subjects who received exchange transfusion or phototherapy (as a way of identifying clinically significant hyperbilirubinemia).

A note is also in order about the exclusion of LDH (lactate dehydrogenase) as a key laboratory value. In the INO-01/ -02 trials, 28 of 30 (93%) of the subjects had an abnormally high LDH on the baseline labs (see 8.1.6.2.1 below). Only one individual started with a normal LDH and had an elevated value on follow-up (subject 01-07010, F/U value 820, upper limits of normal, 800 IU/ml). Of the 28 subjects with elevated LDH at baseline, only 3 had normal or low values at follow-up. Because of the near universal incidence of elevated LDH, both at baseline and on follow-up, it's utility as a marker for individual toxicity is limited. SGOT is the key lab value used in this review to detect hepatocellular injury. Serum SGPT and GGT levels were not collected.

Table 8.1.6.2.2 Number of subjects with normal baseline labs from INO-01/ -02[a].

Laboratory	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
Albumin	18/36 (50%)	18/32 (56%)	16/30 (53%)	16/31 (52%)
Alkaline Phosphatase	16/34 (47%)	12/32 (38%)	19/29 (66%)	19/29 (66%)
BUN	25/38 (66%)	28/40 (70%)	24/31 (77%)	26/34 (76%)
Calcium	15/35 (43%)	14/36 (39%)	20/31 (64%)	21/31 (68%)
Creatinine	21/38 (55%)	21/39 (54%)	17/32 (53%)	18/34 (53%)
Glucose	14/34 (41%)	16/40 (40%)	12/31 (39%)	17/33 (52%)
Phosphate	13/35 (37%)	17/31 (55%)	11/30 (37%)	11/32 (34%)
LDH[b]	2/28 (7%)	5/31 (16%)	1/25 (4%)	2/29 (7%)
SGOT[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%)
Total Protein	19/34 (56%)	12/30 (40%)	9/28 (32%)	12/29(41%)
Uric Acid	26/31 (84%)	19/29 (66%)	19/29 (66%)	19/26 (73%)

a. Data from 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); LDH (lactate dehydrogenase).

8.1.6.2 Standard Analyses and Explorations of Laboratory Data (cont)

Hematology values were examined using the same rule. In this case, platelet count, white blood cell count, and hematocrit were chosen as key lab parameters.

The mechanism for identifying individual subjects of interest was from the adverse events identified by the clinicians. The investigators were instructed to consider a laboratory finding an adverse event if it did not resolve in normal follow-up, or if it altered therapy (personal communication from Dr. Rick Straube, Ohmeda, Inc.). Some of these clearly were not included in the two sets of lab values submitted to the NDA, as evidenced by the 'hyponatremia' adverse event that was recorded (subject 02-15005) and the 'hypokalemia' adverse event that was recorded (subject 01-01009).

The second set of data that was examined for laboratory adverse events relates to a small number of laboratory values which were followed for their perceived clinical relevance: methemoglobin levels; NO_2 levels; and oxygenation parameters. Data for these are available from the datasets of the individual trials, and in summaries from the sponsor within each trial report. For methemoglobin and oxygenation, data was collected in all four of the trials in the NDA. For NO_2 levels, data was collected from all of the trials except the INOSG trial. Both summary and raw data was examined for both changes in the average values, as well as the outliers in both groups. Since elevated levels of NO_2 and methemoglobin led to the discontinuation of substantial numbers of subjects in the 80 ppm I-NO, the case report forms for all of the discontinued subjects was scrutinized.

8.1.6.2.1 Analyses Focused on Measures of Central Tendency

8.1.6. 2.1.1 Lab Values from the INO-01/ -02 study

The table below, and continuing on the next page, summarizes the mean values of the clinical chemistries collected as part of the INO-01/ -02 study. Because of the small numbers of subjects in the INO-03 trail, a summary table of mean clinical and hematology data will not be included. Data from the INO-03 trial will be included in section 8.1.6.2.2, which focuses on an outlier analysis of laboratory data.

No significant differences between the placebo and I-NO groups are evident. There are a number of significant lab changes, which might be expected to follow treatment of acutely ill neonates (increased phosphate, decreased LDH, alkaline phosphatase and SGOT). The increase in mean BUN may reflect both catabolism during acute illness as well as mild renal injury due to hypoxia. Overall, the extreme variability of the infants limits the sensitivity of mean lab values to detect possible adverse events. These changes will be discussed further in section 8.2.

Table 8.1.6.2.1.1.1 Mean clinical chemistry values from INO-01/ -02[a,c].

Lab Test[b]	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	Post-Study gas	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO
Albumin	3±0.5 n=41	3.1±0.6 n=34	3.0±0.5 n=39	2.9±0.5 n=34	2.9±0.5 n=	2.92±0.5 n=30	3.03±0.5 n=35	2.9±0.6 n=33
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
BUN	9.8±5.6 n=41	15±12 n=38	9.7±4.2 n=41	16.3±11 n=40	11±8 n=36	19±18 n=32	8.8±3.1 n=36	12.9±8.9 n=36
Calcium	8.3±1.3 n =41	8.8±1.8 n=35	7.9±1.6 n=40	8.6±1.7 n=37	8.2±1.2 n=34	8.6±1.2 n=31	8.2±1.6 n=34	8.3±1.8 n=34
Creatinine	0.9 ±0.35 n=41	0.9 ±0.56 38	0.89 ±0.21 n=41	0.75 ±0.28 n=40	0.98 ±0.41 n=36	0.96 ±0.74 n=32	0.91 ±0.29 n=35	0.94 ±0.66 n=36
Glucose	109 ±63 n=40	119 ±64 n=36	139 ±149 n=41	109 ±38 n=40	108 ±42 n=36	112 ±55 n=31	102 ±68 n=36	122 ±57 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-I-NO labs differ significantly using 2-sided unpaired t test.

8.1.6. 2.1.1 Lab Values from the INO-01/ -02 study (cont)

Table 8.1.6.2.1.1.1 Mean clinical chemistry values from INO-01/ -02 (cont)[a,c].

Lab Test[b]	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	Post-Study Gas	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO
Phosphate	3.8 ±1.4 n=39	5.2 ±1.8 n=36	3.9 ±1.5 n=37	5.5 ±2.1 n=35	4.2 ±1.5 n=36	5.2 ±1.7 n=31	3.9 ±1.6 n=36	4.6 ±1.5 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
SGOT	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
Total Protein	4.9 ±0.6 n=39	5.0 ±0.9 n=32	4.7 ±0.7 n=36	4.9 ±0.7 n=33	4.8 ±0.6 n=34	4.8 ±0.8 n=34	4.9 ±0.6 n=35	4.7 ±0.6 n=33
Uric Acid	5.9 ±1.9 n=39	4.7 ±3.0 n=32	6.2 ±1.9 n=38	3.9 ±1.8 n=30	7.2 ±2.6 n=35	5.3 ±3.5 n=29	6.8 ±2.8 n=35	4.9 ±2.5 n=30

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-I-NO labs differ significantly using 2-sided unpaired t test.

The table below summarizes the mean values of the clinical hematologies collected as part of the INO-01/ -02 study. Because of the small numbers of subjects in the INO-03 trail, a summary table of mean clinical and hematology data will not be included. Data from the INO-03 trial will be included in section 8.1.6.2.2, which focuses on an outlier analysis of laboratory data. Note the decreased immature neutrophils in the I-NO groups.

Table 8.1.6.2.1.1.2 Mean hematology values from INO-01/ -02[a,c].

Lab Test[b]	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	Post-Study Gas	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO
RBC # (10^6 cells/ml)	4.4 ±0.8 n=41	4.5 ±0.6 n=38	4.6 ±0.7 n=41	4.4 ±0.7 n=41	4.2 ±0.9 n=36	4.4 ±0.6 n=34	4.3 ±0.9 n=37	4.5 ±0.6 n=36
Total hemoglobin (mg/dl)	15.5 ±2.6 n=41	14.9 ±1.9 n=38	15.9 ±2.4 n=41	14.7 ±2.3 n=41	15.1 ±2.7 n=36	15.0 ±2.1 n=34	15.4 ±2.7 n=37	14.9 ±1.9 n=36
Hematocrit (%)	46.3 ±7.8 n=41	43.9 ±5.3 n=38	47.3 ±7.1 n=41	43.5 ±6.7 n=41	44.8 ±8.4 n=38	43.4 ±6.1 n=34	44.3 ±10.5 n=37	44.0 ±5.7 n=36
WBC # (10^6 cells/ml)	16.3 ±7.0 n=41	12.9 ±6.3 n=38	15.5 ±7.5 n=41	11.5 ±5.2 n=41	15.8 ±11.3 n=36	11.2 ±5.5 n=34	18.8 ±7.7 n=37	13.6 ±6.2 n=36
Lymphocytes (% of WBCs)	22.6 ±12 n=41	26.6 ±12 n=37	27.7 ±20 n=41	26 ±11 n=40	23.5 ±13 n=36	29 ±9 n=32	22 ±12 n=36	27 ±13 n=36
Neutrophils (% of WBCs)	54 ±18 n=41	50 ±19 n=37	53 ±22 n=41	54 ±14 n=40	50 ±19 n=36	49 ±12 n=32	56 ±14 n=35	56 ±16 n=35
Immature neutrophils (% of WBCs)	16 ±12 n=41	13 ±16 n=38	12 ±9.5 n=41	8.4 ±9.8 n=39	16.4 ±13 n=36	9.8 ±10 n=31	14.1 ±10 n=36	7.9 ±8.3 n=35
Monocytes (% of WBCs)	4.6 ±2.8 n=41	5.9 ±4.1 n=37	4.9 ±3.5 n=41	7.4 ±4.9 n=40	6.9 ±5.7 n=36	7.2 ±4.8 n=32	5.3 ±4.1 n=36	5.4 ±4.5 n=36
Basophils (% of WBCs)	0.1 ±0.4 n=40	0.1 ±0.3 n=34	0.2± 0.5 n=40	0.7 ±3.0 n=37	0.2 ±0.5 n=36	0.3 ±0.7 n=32	0.2 ±0.5 n=34	0.4 ±0.7 n=33
Eosinophils (% of 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
Platelet count	195 ±80 n=41	185 ±92 n=38	190 ±68 n=41	170 ±68 n=41	187 ±77 n=36	170 ±82 n=34	183 ±73 n=37	177 ±92 n=35

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). Shaded boxes indicate that baseline and post-I-NO labs differ significantly using 2-sided unpaired t test.

8.1.6. 2.1.2 Mean peak NO_2 concentrations.

8.1.6.2.1.2a NO_2 concentrations from the NINOS trial.

Table 8.1.6.2.1.2a.1 Peak NO_2 levels in ppm from the NINOS trial[a].

	Control	I-NO	p value[b]
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

a. Data from electronic datasets and NDA volume 2.14.
b. p value using Student's t-test or chi-square as appropriate.

8.1.6.2.1.2b NO_2 concentrations from the INOSG trial

In the INOSG trial no data on NO_2 levels were collected.

8.1.6.2.1.2c NO_2 concentrations from 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 NO2 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 at any time during the trial were in the 80 ppm group.

Table 8.1.6.2.1.2c.1 Peak NO_2 levels in ppm from the INO-01/ -02 trial[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
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%)

a. Data from electronic datasets, and NDA volume 2.26.

8.1.6.2.1.2d NO_2 concentrations from the INO-03 trial

Despite the small numbers of subjects in the INO-03 trial, there was a clear pattern of significantly higher peak NO_2 values in the 80 ppm group.

Table 8.1.6.2.1.2d.1 Peak NO_2 levels from the INO-03 trial[a].

	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
Peak NO_2 level at any time	0.52±0.55	0.48±0.63	2.45±0.92
Peak NO_2 level at any time (ppm)			
0.0 - 1.0	4/4 (100%)	7/8 (88%)	0/2 (0%)
1.1 - 3.0	0/4 (0%)	1/8 (12%)	1/2 (50%)
3.0- 5.0	0/4 (0%)	0/8 (0%)	1/2 (50%)
5.1 - 7.0	0/4 (0%)	0/8 (0%)	0/2 (0%)
7.1 to 10	0/4 (0%)	0/8 (0%)	0/2 (0%)

a. Data from NDA volume 2.31.

8.1.6.2.1.3 Methemoglobin concentrations.

8.1.6.2.1.3a Methemoglobin levels from the NINOS trial

In the NINOS trial, subjects receiving I-NO had a higher average methemoglobin concentration, and a higher peak methemoglobin level.

Table 8.1.6.2.1.3a.1 Peak methemoglobin levels from the NINOS trial[a].

	Control	Combined I-NO	p value[b]
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. Data from electronic datasets and NDA volume 2.14.
b. p value using Student's t-test or chi-square as appropriate.

8.1.6.2.1.3b Methemoglobin levels from the INOSG trial

In the INOSG trial, methemoglobin levels were collected less rigorously than in the INO-01/ -02 /-03 and NINOS trials. In the INOSG study the 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 11 subjects with available data. It is clear that in this trial, using 80 ppm I-NO, a higher % of subjects had markedly elevated methemoglobin levels compared with NINOS. (note: data after 20 minutes is only available for infants in the I-NO group who responded to the I-NO.

Table 8.1.6.2.1.3b.1 Peak Methemoglobin levels from the INOSG trial[b].

	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.
b. Data from NDA volume 2.16.

8.1.6.2.1.2c Methemoglobin levels from the INO-01/ -02 trial

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 8.1.6.2.1.3c.1 Peak methemoglobin levels from the INO-01/ -02 trial (not all subjects had data available)[a].

	Control	I-NO			Combined I-NO
		5 ppm	20 ppm	80 ppm	
Peak methemoglobin level at any time	0.7±0.42	0.89±0.88	1.16±0.69	5.77±2.8 [b]	2.57±2.8[b]
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 (3.5%)
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	2/37 (5%)	2/113 (2%)

a. Data from electronic datasets, and NDA volume 2.26.
b. Differ from control significantly using unpaired t test.

8.1.6.2.1.3d Methemoglobin levels from the INO-03 trial (from available subjects)

Table 8.1.6.2.1.3d.1 Peak Methemoglobin levels from the INO-03 trial[b].

	I-NO		
	5 ppm	20 ppm	80 ppm
Average peak methemoglobin level at any time[a]	1.05±0.58	1.44±0.79	3.6±2.83
Peak methemoglobin level at any time (%)			
0.0 - 1.0	2/4 (50%)	3/8 (38%)	0/2 (0%)
1.1 - 2.0	2/4 (50%)	3/8 (38%)	1/2 (50%)
2.1 - 30	0/4 (0%)	2/8 (25%)	0/2 (0%)
3.1 - 5.0	0/4 (0%)	0/8 (0%)	0/2 (0%)
5.1 to 10	0/4 (0%)	0/8 (0%)	1/2 (50%)

a. Due to small sample sizes, no statistical analysis between groups is performed.
b. Data from NDA volume 2.31.

8.1.6.2.2 Analyses Focused on Outliers

8.1.6.2.2. Laboratory values from the INO-01/ -02 and INO-03 trials

The table below shows the incidence of abnormal lab values in the four treatment groups. The first tables show the incidence of abnormal values for key chemistries and hematologies. The normal values for each of the chemistries varied. This was related both to the laboratory that performed the assay as well as the age of the infant when the blood was drawn. For example, the normal range for bilirubin change from day to day during the first few days of life. They are also affected by pre-maturity. Thus, the normal ranges for bilirubin quoted in the laboratory section (Appendix 16.2.2.13) have a normal range of total bilirubin from 0.2 to 16 mg/dl under certain circumstances. To account for this, each lab value was looked at individually, and the normal range associated with that individual specimen and subject served to determine whether the lab was abnormal or not.

Table 8.1.6.2.2.1 Abnormal key chemistry values from the INO-01/ -02 and INO-03 trials[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 Glucose					
New abnormalities[a]	6 (16%)	5 (11%)	4 (10%)	9 (24%)	18 (15%)
New or worsening abnormalities[b]	13 (34%)	11 (24%)	12 (29%)	14 (38%)	37 (30%)
Low Glucose					
New abnormalities[a]	1 (3%)	1 (2%)	0 (0%)	0 (0%)	1 (<1%)
New or worsening abnormalities[b]	1 (3%)	1 (2%)	0 (0%)	0 (0%)	1 (<1%)
Elevated Total Bilirubin					
New abnormalities[a]	1 (3%)	4 (9%)	1 (2%)	1 (3%)	6 (5%)
Values >12[c]	3 (8%)	4 (9%)	5 (12%)	3 (8%)	12 (10%)
Elevated SGOT					
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 BUN					
New abnormalities[a]	5 (13%)	7 (16%)	10 (24%)	6 (16%)	23 (19%)
New or worsening abnormalities[b]	6 (16%)	10 (22%)	11 (27%)	8 (22%)	29 (24%)
Creatinine					
New abnormalities[a]	3 (8%)	4 (9%)	4 (10%)	3 (8%)	11 (9%)
New or worsening abnormalities[b]	12 (32%)	12 (27%)	10 (24%)	10 (27%)	32 (26%)

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.

8.1.6.2.2. Laboratory values from the INO-01/ -02 and INO-03 trials (cont)

Table 8.1.6.2.2.2 Miscellaneous key chemistry values from the INO-01/ -02 and INO-03 trials.

	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 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%)
Low Calcium					
New abnormalities[a]	2 (5%)	3 (7%)	3 (7%)	6 (16%)	12 (10%)
New or worsening abnormalities[b]	5 (13%)	8 (18%)	4 (10%)	11 (30%)	23 (14%)
High Calcium					
New abnormalities[a]	0 (0%)	1 (2%) -	0 (0%)	1 (3%)	2 (2%)
New or worsening abnormalities[b]	4 (10%)	2 (4%)	1 (2%)	1 (3%)	4 (3%0
Low Albumin					
New abnormalities[a]	7 (18%)	4 (9%)	7 (17%)	5 (14%)	16 (13%)

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.

Table 8.1.6.2.2.3 Abnormal key hematology values from the INO-01/ -02 and INO-03 trials[c].

	Control n =38	I-NO 5 ppm n = 41	I-NO 20 ppm n =32	I-NO 80 ppm n = 38	I-NO Combined n=111
Low Platelet Count					
New abnormalities[a]	8 (21%)	13 (32%)	9 (28%)	5 (13%)	27 (24%)
New or worsening abnormalities[b]	14 (37%)	13 (32%)	12 (38%)	10 (26%)	35 (32%)
Low WBC Count					
New abnormalities[a]	3 (8%)	3 (7%)	4 (12%)	2 (5%)	9 (8%)
New or worsening abnormalities[b]	3 (8%)	4 (10%)	4 (12%)	3 (8%0	11 (10%)
Low RBC Count					
New abnormalities[a]	6 (16%)	9 (22%)	6 (19%)	7 (18%)	22 (20%)
New or worsening abnormalities[b]	11 (29%)	12 (29%)	7 (22%)	9 (24%)	28 (25%)

a. These subjects had a normal value at baseline and an abnormally low 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 abnormally low value at baseline which was lower on the follow-up lab.

c. Data was obtained from NDA volume 2.31, Data Listing 13.2; volume 2.25, Appendix 16.2.2.13; and volume 2.18, Table T-30, and electronic datasets.

8.1.6.2.2. Laboratory values from the INO-01/ -02 and INO-03 trials

Table 8.1.6.2.2.4 Miscellaneous hematology values from the INO-01/ -02 and INO-03 trials[c].

	Control n =38	I-NO 5 ppm n = 41	I-NO 20 ppm n =32	I-NO 80 ppm n = 38	I-NO Combined n=111
High Eosinophil Count					
New abnormalities[a]	4 (10%)	6 (15%)	6 (19%)	6 (16%)	18 (16%)
New or worsening abnormalities[b]	4 (10%)	7 (17%)	6 (19%)	7 (18%)	20 (18%)
Low Hematocrit					
New abnormalities[a]	6 (16%)	8 (20%)	6 (19%)	5 (13%)	19 (17%)
New or worsening abnormalities[b]	12 (32%)	12 (29%)	10 (31%)	8 (21%)	30 (27%)

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 13.2; volume 2.25, Appendix 16.2.2.13; and volume 2.18, Table T-30, and electronic datasets.

8.1.6.2.2.1 Individual subjects with outlier lab values identified in INO-01/ -02 and INO-03 trials

8.1.6.2.2.1a Individual subjects with outlier lab values identified from submitted lab data

The two tables below identify the specific subjects and lab tests which were identified as markedly abnormal (>2X upper limits of normal or <0.5X lower limit of normal on post-I-NO lab). Specific cases from these tables will be discussed in section 8.2 in the appropriate body system.

The first table shows the abnormal chemistry lab values.

Table 8.1.6.2.2.1a.1 Individuals with markedly abnormal post-I-NO chemistry labs from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo				
01-03004	Phosphate	4.6	2.3	low
01-03006	Phosphate	3.7	1.4	low
01-03010	Phosphate	3.0	2.1	low
01-03013	LDH	550	720	high
01-04001	LDH	515	527	high
02-14004	Bilirubin	9.3	14.3	high
01-14002	Bilirubin	9	17.2	high
01-07007	Bilirubin	10.5	15	high
01-17002	Glucose	71	191	high
01-07010	Glucose	140	189	high
01-07001	Creatinine	1.2	1.9	high
I-NO 5 ppm				
01-03002	LDH	517	939	high
01-06002	LDH	1955	3981	high
02-14001	LDH	530	1235	high
02-15001	LDH	510	1022	high
01-01004	Bilirubin	5.5	13.7	high
02-11004	BUN	8.7	18.2	high
02-11008	SGOT	78	145	high

a. Data from NDA, volumes 2.25 and 2.31, individual patient listings

b. Lab tests were identified as markedly abnormal were >2X upper limits of normal or <0.5X lower limit of normal on post-study gas value. Units are not included in the table but are the standard per the sponsor for each test.

8.1.6.2.2.1a Individual subjects with outlier lab values identified from submitted lab data (cont)

Table 8.1.6.2.2.1a.1 Individuals with markedly abnormal post-I-NO chemistry labs from INO-01/ -02 and /-03 trials (cont)[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
I-NO 20 ppm				
01-17006	LDH	2939	3946	high
01-03020	Phosphate	2.7	2.5	low
01-07003	Bilirubin	5.9	14.2	high
01-07005	Bilirubin	12.9	13.9	high
01-09003	Bilirubin	18.9	30.1	high
01-14001	Bilirubin	9.5	14.1	high
03-52001	Bilirubin	11	15.3	high
01-03015	Glucose	135	279	high
01-11001	Glucose	190	288	high
01-03025	SGOT	109	358	high
01-03008	SGOT	181	264	high
01-03001	Creatinine	0.9	1.6	high
01-03008	Creatinine	0.8	4.2	high
01-03025	BUN	12	59	high
01-07005	BUN	17	90	high
03-67001	BUN	9	45	high
03-67002	Uric Acid	5.1	1.6	low
I-NO 80 ppm				
01-03003	LDH	475	692	high
01-06003	LDH	1936	2995	high
01-11004	LDH	3429	6270	high
02-04004	LDH	508	1098	high
01-02003	LDH	763	1623	high
01-03005	Bilirubin	9.8	13.5	high
01-05003	Bilirubin	10.9	13.4	high
02-13001	Bilirubin	9.4	13.1	high
02-11007	LDH	1534	2517	high
03-59003	LDH	NA	1634	high
01-04005	Glucose	92	320	high
02-06001	Glucose	98	217	high
02-07003	Glucose	70	166	high
01-06003	Glucose	77	258	high
01-02003	SGOT	69	120	high
01-03029	Creatinine	1.1	3.5	high

a. Data from NDA, volumes 2.25 and 2.31, and individual patient listings.

b. Lab tests were identified as markedly abnormal were >2X upper limits of normal or <0.5X lower limit of normal on post-study gas value. Units are not included in the table but are the standard per the sponsor for each test.

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8.1.6.2.2.1a Individual subjects with outlier lab values identified from submitted lab data

The next table lists the individuals with markedly abnormal post-study gas labs from the INO-01/ -02 and /-03 trials. Platelet and WBC count are expressed in numbers of thousands.

Table 8.1.6.2.2.1a.2 Individuals with markedly abnormal post-I-NO hematology labs from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo				
01-09001	WBC	38.7	23	high
01-03019	Bands	31	53	high
01-11002	Bands	26	52	high
02-12002	Bands	30	48	high
01-11008	Neutrophils	66	71	high
01-11002	Lymphocytes	23	6	low
02-04001	Lymphocytes	14	10	low
01-08001	Eosinophils	1	8	high
01-06008	Platelets	40	57	low
01-17002	Platelets	197	70	low
I-NO 5 ppm				
01-05002	RBC	3.8	2.9	low
01-02002	Bands	21	34	high
01-03024	Bands	17	32	high
01-07002	Bands	18	32	high
01-17005	Bands	16	2	low
02-14002	Bands	37	16	high
02-17002	Bands	12	1	low
03-57002	Neutrophils	72	60	high
01-02002	Neutrophils	7	12	low
01-11012	Lymphocytes	13	13	low
01-04007	Monocytes	0	1	low
01-10002	Eosinophils	0	14	high
01-11012	Eosinophils	0	7	high
01-03014	Platelets (x1000)	171	69	low

a. Data from NDA, volumes 2.25 and 2.31, individual patient listings

b. Lab tests were identified as markedly abnormal were >2X upper limits of normal or <0.5X lower limit of normal on post-study gas value. Units are not included in the table but are the standard per the sponsor for each test.

8.1.6.2.2.1a Individual subjects with outlier lab values identified from submitted lab data (cont)

The table below continues the list of markedly abnormal hematology lab values.

Table 8.1.6.2.2.1a.2 Individuals with markedly abnormal post-I-NO hematology labs from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
I-NO 20 ppm				
01-09003	WBC	70.7	23	high
02-07007	Bands	25	22	high
03-58001	Bands	8	16	high
03-59002	Bands	23	2	low
01-03001	Bands	31	40	high
01-03011	Bands	45	32	high
01-07003	Bands	1	1	low
02-04002	Monocytes	2	0	low
02-11006	Monocytes	3	9	high
03-67002	Monocytes	1	13	high
03-67002	Eosinophils	1	17	high
01-10001	Platelets	219	64	low
01-11001	Platelets	164	10	low
I-NO 80 ppm				
03-57001	Neutrophils	59	61	high
01-07004	Bands	15	25	high
02-07003	Bands	8	16	high
02-07005	Bands	15	18	high
01-11013	Lymphocytes	23	17	low
02-07003	Lymphocytes	28	6	low
02-15003	Lymphocytes	14	9	low
01-03018	Monocytes	2	0	low
02-11003	Monocytes	11	10	high
02-11007	Monocytes	8	9	high
02-12001	Monocytes	4	0	low
02-11007	Eosinophils	5	22	high
01-05005	Eosinophils	2	9	high
02-15006	Eosinophils	1	11	high
01-07004	Platelets (x1000)	70	46	low
02-07005	Platelets	166	64	low

a. Data from NDA, volumes 2.25 and 2.31, individual patient listings

b. Lab tests were identified as markedly abnormal were >2X upper limits of normal or <0.5X lower limit of normal on post-study gas value. Units are not included in the table but are the standard per the sponsor for each test.

8.1.6.2.2.1b Individual subjects with outlier lab values identified as adverse events by individual investigators from the INO-01/ INO-02 and -03 trials

The next method used in this review to identify 'outlier' subjects was to examine the subjects identified by the individual investigators as having adverse events related to lab values. Notes and outcomes were derived from sponsor information or reviewer examination of case report forms and electronic datasets. Information regarding follow-up of any lab marked as 'continuing' was specifically requested from the sponsor, but was not available at the time of the NDA withdrawal.

Table 8.1.6.2.2.1b.1 Adverse events related to lab measurements, identified by the individual study investigators[a].

Patient #	Adverse Event/ Lab Test	Notes/ Outcomes
Placebo		
01-06008	Anemia	Recovered, duration 8 hours
02-07008	Thrombocytopenia	Recovered, duration 1 day
01-15001	Bilirubinemia	Recovered, duration 7 days
02-15004	Bilirubinemia	Recovered, duration 2 days
01-01009	Hypokalemia	Recovered, duration 1 day
02-15005	Hyponatremia	Recovered, duration 2 days
02-15005	Kidney Failure	Recovered, duration 3 days
I-NO 5 ppm		
01-06009	Bilirubinemia	Recovered, duration 2 days
01-14003	Bilirubinemia	Recovered, duration 6 hours
02-11004	Bilirubinemia	Continuing
02-11008	Bilirubinemia	Continuing
02-11008	Hypocalcemia	Recovered, duration 2 days
01-01008	Kidney Tubular Necrosis	No information available
I-NO 20 ppm		
02-07007	Bilirubinemia	Recovered, duration 18 days
02-15002	Bilirubinemia	Recovered, duration 2 days
03-67001	Bilirubinemia	'Recovered, duration 3 days'
02-07007	Hemorrhage	Improved, duration 3 hours
03-67002	Anemia, iatrogenic	'Recovered, duration 1 day'
03-62001	Anemia	'Recovered, duration 9 days'
01-17006	Thrombocytopenia	Recovered, duration 2 days
03-62001	Thrombocytopenia	Recovered, duration 5 days
03-58001	Thrombocytopenia	Improved, duration 15 hours
01-03025	Kidney Failure	Required therapy, subject ultimately died
03-67002	Kidney Failure	Recovered, duration 3 days'
03-59005	Hypocalcemia	Recovered
03-67002	Calcium disorder	Improved, duration continuing
03-59005	Hypokalemia	Recovered, duration 2 hours'
03-67002	Hyperglycemia	Recovered, duration 8 hours

a. Subject data comes from NDA, volume 2.17 (Table 41) and 2.31 (Data listing 14): Listing of Adverse Events from INO-01/ INO-02 and INO-03 and individual case report forms. No individual lab data was available for these adverse events, with the exception of the methemoglobins, which are discussed below.

8.1.6.2.2.1b Individual subjects with outlier lab values identified as adverse events by individual investigators from the INO-01/ INO-02 and -03 trials

The table below continues the abnormal lab values identified by the individual investigators

Table 8.1.6.2.2.1b.1 Adverse events related to lab measurements, identified by the individual study investigators (cont) [a].

Patient #	Adverse Event/ Lab Test	Notes/ Outcomes
I-NO 80 ppm		
01-01002	Bilirubinemia	Prolonged hospitalization, event continues 'Possible' relationship to drug per investigator
01-01002	Bilirubinemia	Continuing,, unchanged
02-15003	Bilirubinemia	Recovered, duration 4 days
02-04006	Hemorrhage	Continuing, unchanged
02-04004	Anemia	Recovered, duration 2 days
01-04005	Hyponatremia	Recovered, duration 10 hours
02-15003	Hypoglycemia	Recovered, duration 4 hours
03-59003	Thrombocytopenia	'Recovered, duration 2 days'
01-01005	Methemoglobinemia	Recovered, duration 40 minutes
01-03003	Methemoglobinemia	Recovered, duration 4 hours
01-03016	Methemoglobinemia	Recovered, duration 8 hours
01-03029	Methemoglobinemia	Recovered, duration 8 hours
01-05005	Methemoglobinemia	Recovered, duration 2 hours
01-06003	Methemoglobinemia	Recovered, duration 2 hours
01-06006	Methemoglobinemia	Recovered,. duration 18 hours
01-11004	Methemoglobinemia	Recovered, duration 2 hours
01-17004	Methemoglobinemia	Recovered, duration 5 hours
02-04004	Methemoglobinemia	Recovered, duration 1 hours
02-07003	Methemoglobinemia	Recovered, duration 6 hours

a. Subject data comes from NDA, volume 2.17 (Table 41) and 2.31 (Data listing 14): Listing of Adverse Events from INO-01/ INO-02 and INO-03 and individual case report forms. No individual lab data was available for these adverse events, with the exception of the methemoglobins, which are discussed below.

8.1.6.2.2.1 Abnormal NO_2 levels from the NINOS, INO-01/ -02 and /-03 trials

The next 'outlier' lab population to be explored are those subjects with elevations in either methemoglobin or NO_2. All subjects identified as having elevations in either lab value by the sponsor and after review of all individual lab values are included in the sections below, and are arranged by study.

Of the infants identified with increased NO_2 concentrations, two infants received ECMO, and one of those infants died. One of the infants had chronic pulmonary disease. The infant that died required discontinuation of I-NO due to elevated methemoglobin levels, and died several days after discontinuation. Overall, no short-term adverse outcomes were identified that can be linked to the elevated NO_2 levels. Additionally, no link between NO_2 concentrations and chronic pulmonary disease is apparent.

8.1.6.2.2.1a Elevated NO_2 levels from the NINOS trial

In the NINOS trial, one subject receiving I-NO 80 ppm had a NO_2 level >7.0 ppm during the trial.

1. Subject #55-08, a Caucasian male, had a peak level of 9.1 ppm, and the subject underwent a successful wean of study gas, without withdrawal of the subject from the trial. He received ECMO and was discharged home with chronic lung disease.

8.1.6.2.2.1b Elevated NO_2 levels from the INOSG trial

In the INOSG trial no data on NO_2 levels were collected.

8.1.6.2.2.1c Elevated NO_2 levels from the INO-01/ -02 trial

Control Group

There were 5 individuals in the control group who had NO_2 levels of >2.0 ppm during the trial. Three of these individuals had levels which were >2.0 for at least two consecutive periods (30-60 minutes apart).

1. Subject #01-03004 had an NO_2 level of 2.0 at 30 and 60 minutes of study gas, did not respond and was withdrawn. The subject received ECMO, but did not die, and was discharged with no evidence of chronic lung disease.

2. Subject #01-07007, a Caucasian female, had an NO_2 level which ranged between 1.0 and 3.0 on control gas for the duration of therapy (30 minutes to 48 hours). She had no major adverse events (ECMO, chronic lung disease, death).

3. Subject #02-11001, a Caucasian female, had an NO_2 level which was 0.0 for the majority of the exposure to study gas (148 hours). During the period from 112 to 124 hours the NO_2 level was 2.0 to 3.0 (4 measurements), and measured 3.0 at hour 124. The infant was discharged home without receiving ECMO, and with no chronic pulmonary disease.

I-NO 5 ppm Group

There was one individual, an African-American female in the I-NO 5 ppm group (#02-04003), who had NO_2 =3.0 ppm for 3 consecutive measurements after 8-12 hours of study gas. She had no major adverse events (ECMO, chronic lung disease, death).

I-NO 80 ppm Group

In the INO-01/ -02 trial, three subjects who received I-NO, 80 ppm, developed NO_2 levels > 5ppm.

1. Subject #01-04005 had NO_2 levels of 6.2 and 5.9 after 1 and 3 hours. His I-NO was tapered to 40 ppm and his NO_2 levels fell. His maximum methemoglobin level was 2.5 %. He did not require ECMO, and was discharged with a seizure disorder but not chronic lung disease.

2. Subject #01-06006 had an NO_2 level of 5.0 after 1 hour. Her NO_2 levels fell to 2.8 ppm without adjustment of I-NO concentration. However, her methemoglobin levels continued to rise (7.4 % after 8 hours), and I-NO was tapered off. She developed a series of pneumothoraces and ultimately died after receiving ECMO.

3. Subject #02-15006 had an NO_2 level of 6.2 after 30 minutes. The repeat value was 1.4 and no change in I-NO concentration was necessary. The infant was discharged home without receiving ECMO, and with no chronic pulmonary disease.

8.1.6.2.2.1d Elevated NO_2 levels from the INO-03 trial

In the INO-03 trial, only one infant had NO_2 levels>3.0 ppm.

1. Subject #03-59003, an African-American male, had NO_2 levels >2.9 for the first 12 hours of I-NO (80ppm), with a peak of 3.3 ppm. His treatment gas was then lowered to 70 ppm for approximately 50 hours, during which time his NO_2 levels fell to between 1.9 and 2.1. When he was returned to I-NO 80 ppm, his NO_2 levels remained <3. He was deemed a treatment failure due to the high NO_2 level, but did not receive ECMO, and was discharged home with chronic lung disease. His hospitalization was also complicated by hypophosphatemia requiring therapy.

8.1.6.2.2.2 Methemoglobin levels

8.1.6.2.2.2a Elevated methemoglobin levels from the NINOS trial

The sponsor also identified increased levels of methemoglobin as an adverse event, and in both the NINOS and INO-01/ -02 trials was a reason to decrease and/or discontinue the study gas.

In the NINOS trial, methemoglobin was defined as >5%, and a total of 11 subjects (4 controls, 7 I-NO) had their study gas decreased because their methemoglobin levels exceeded this level. All of these subjects continued on study gas at lower flow rate. No subject was discontinued because of NO_2 >7 ppm or methemoglobin >10%.

8.1.6.2.2.2b Elevated methemoglobin levels from the INOSG trial

Three of the infants had peak methemoglobin levels >5% during the trial (the criteria for elevated methemoglobin specified in the trial).

1. Subject #UT@D-1 was a female infant who responded to I-NO, 80 ppm, with a decrease in OI. Her methemoglobin level was 13.2 after 12 hours, and 8.7 after 24 hours. That is the last recorded methemoglobin level, and the infant later died without receiving ECMO.

2. Subject #UCSF-5, had a methemoglobin level of 18.2% at 24 hours of I-NO therapy. 'Because the patient's oxygenation had improved, inhaled NO was continued.' (NDA volume 2.16, page 060908). The I-NO was decreased from 80 to 10 gradually, and the methemoglobin level declined over time to 1.3% after 144 hours. The infant survived without ECMO and no recorded pulmonary disease.

3. Subject #Buf-S1 had a methemoglobin level of 9.0 after 24 hours on I-NO 40-80 ppm. Reduction of the I-NO to 10 ppm reduced the level to 1.5 after 108 hours. The infant was weaned successfully, and survived without receiving ECMO and without known pulmonary disease.

8.1.6.2.2c Elevated methemoglobin levels from the INO-01/ -02 trial

In the INO-01/ -02 trial, thirteen subjects, all in the I-NO 80 ppm group, were 'treatment failures' as the result of methemoglobin levels >7%. This represents 35% of the 80 ppm group. A listing of these infants can be found in the table below, including outcome data for ECMO, ventilation therapies, neurologic disorders, and chronic lung disease (CLD). For those infants who dropped from study as a result of elevated methemoglobin levels, their narratives are in section 8.1.3.2.1 above.

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.

Of the infants identified with increased methemoglobin concentrations, two infants received ECMO, and one of those infants died. None of the infants had chronic lung disease (CLD). The infant that died required discontinuation of I-NO due to elevated methemoglobin levels, and died several days after discontinuation. Overall, no short-term adverse outcomes were identified that can be linked to the elevated methemoglobin levels. Specifically, no link between methemoglobin concentrations and chronic pulmonary disease is apparent.

Table 8.1.2.2c.1 Subjects from the INO-01/ -02 and INO-3 trials who had elevated methemoglobin levels.

Study Group	Subject #	Peak methemoglobin level	Time to Peak	Outcome
I-NO 80 ppm				
	01-01005	7.2%	40	No ECMO Discharged without CLD
	01-03003	7.3%	16	No ECMO Discharged without CLD
	01-03016	8.4%	4	No ECMO Discharged without CLD
	01-03029	11.9%	8	No ECMO Discharged without CLD
	01-04005	7.4%	8	No ECMO Discharged without CLD, with seizures
	01-05005	7.6%	12	No ECMO Discharged without CLD (some F/U data missing)
	01-06003	8.4%	8	No ECMO Received HFOV Discharged without CLD (some F/U data missing)
	01-06006	7.3%	9	Died No ECMO
	01-11004	8.4%	8	No ECMO Discharged without CLD Had seizure disorder
	01-17004	9.3%	8	No ECMO Discharged without CLD
	02-04004	9.5%	4	No ECMO Discharged without CLD
	02-04006	10.8%	4	Received ECMO Discharged without CLD
	02-07003	7.8%	8	No ECMO Discharged without CLD

a. Data comes from review of individual case report forms, NDA volumes 3.4-3.10 and from electronic datasets.

8.1.6.2.2d Methemoglobin levels from the INO-03 trial

In the INO-03 trial, no subject (of 14) had methemoglobin level >7%.

8.1.6.3 Dropouts for Laboratory Abnormalities

The only dropouts for laboratory values that were identified in the NDA were those subjects who had elevations in methemoglobin and NO_2. These have been previously discussed.

8.1.6.4 Additional Analyses and Explorations

8.1.6.4.1 Analysis of outcomes by sex and race

With the following exceptions, the number of subjects who reported a given laboratory related adverse event in the database is too small for meaningful analysis by race, sex and age.

Analysis of methemoglobin levels by race was performed above, and no racial interaction was identified.

The table below shows the demographics of the subjects who had markedly abnormal labs (Tables 8.1.6.2.2.1a.1 and 8.1.6.2.2.1a.2), as well as the 13 subjects with methemoglobinemia (from Table 8.1.3.2.1.1). The only striking pattern from the summary above is the small number of white males who had elevated methemoglobin levels. In the INO-01/ -02 and /-03 trials, they made up approximately 50% of the subjects in the 80 ppm group, and yet comprise only 8% of the subjects with methemoglobin identified as an adverse event.

Table 8.1.6.4.1.1 Demographics of abnormal laboratory measurements from INO-01/ -02

Laboratory	Control	Pooled I-NO
Elevated eosinophil count	1 WM	4 WM 2 WF
Decreased platelet count	2 WM	3 WM 1 Hispanic F 1 BM
Decreased lymphocyte count	1 WM	3 WM 1 BM 1 Other M
Elevated glucose	1 BF 1 WF	2 WM 2 BM 1 BF 1 Hispanic M 1 Hispanic F
Elevated methemoglobin	No subjects	1 WM 3 BM 3 Hispanic M 3 WF 2 BF 1 Other F

8.1.6.4.2 Drug-drug interactions

Given the small number of subjects in the available database with information regarding concomitant medications, no useful analysis for drug-drug interactions is possible.

8.1.7 Vital Signs

8.1.7.1 Vital sign collection in the four trials

The effect of I-NO on two sets of vital signs will be examined: blood pressure and heart rate. Because the infants were intubated, and largely paralyzed, data on the rate of respiration will not be examined.

Examination of the effects of I-NO on systemic BP and vital signs is divided into acute measurement of the effects of I-NO (0 to 30 minutes) and the chronic effects of I-NO (beyond 30 minutes), based on the data available for examination.

The INO-03 trial collected no control data. The INO-01/ -02 trial collected data at baseline and again after 30 minutes of exposure to study gas. The INOSG trial collected data at baseline and 20 minutes. Each study will be examined in turn. Hemodynamic data was not collected after administration of study gas as part of the NINOS trial.

The table below summarizes the data available for the acute effects of study gas on BP and heart rate.

Table 8.1.7.1.1 Short-term data available on vital signs from the INOSG and INO-01/ -02 trials[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
INOSG	25			28	28
INO-01/ -02	41	41	36	35	112
Total	66	41	36	63	140

a. Data from NDA volumes 2.16, 2.18, 2.31, and electronic datasets.

For the long-term effects of I-NO on systemic BP and heart rate, the data comes primarily from the INO-01/ -02, for which recorded values are available from baseline to the end of exposure to I-NO for all subjects. The number of subjects with available data at baseline is shown below. As individuals were withdrawn from study gas, the number of subjects in each group fell, and the number of subjects after 24, 48, and 120 hours is quite small. While the INOSG trial collected data on the sub-set of subjects responding to I-NO, no control data of any kind was collected.

Table 8.1.7.1.2 Data available for the vital signs of individual subjects at various times after exposure to study gas from INO-01/ -02 trial.

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
Baseline	41	41	36	35	112
0.5 hours	41	41	36	35	112
12 hours	26	29	25	26	70
24 hours	23	24	19	12	55
48 hours	17	17	15	7	39
72 hours	15	15	11	4	30
96 hours	11	10	7	3	20
120 hours	9	8	3	2	13

a. Data from NDA, volume 2.18, Table T-8, T-9, and T-10, as well as volume 2.23 and the electronic dataset.

8.1.7.2 Acute effects of I-NO on vital signs

The effect of I-NO on heart rate and BP will be examined separately.

8.1.7.2a Acute effects of I-NO on blood pressure

Two placebo-controlled trials measured the acute effects of I-NO on BP and heart rate, and those results are shown below. No significant difference between the control group (N_2) and the I-NO group was seen in the INO-01/ -02 trial were seen. In the INOSG trial, a small but significant decrease in the diastolic, systolic and mean blood pressures was reported by the sponsor when the first baseline value, taken before the FiO_2 was reduced to 90%, was compared with the 20 minute value. The values below compare the change from the second baseline value, taken after the FiO_2 was reduced, to the 20 minute value. No significant differences in the latter group were detected.

Table 8.1.7.2a.1 Acute effects of I-NO on blood pressure in the INOSG and INO-01/ -02 trials[c].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
Total subjects	66	41	36	63	140
Mean systemic arterial pressure (mmHg)					
INOSG[b]					
Baseline	55.6±10			56±10	
20 minutes of study gas	57.2±12			55.0±10	
INO-01/ -02[a]					
Baseline	54.5±10	54.5±8	53.5±13	49.5±9	52.6±10
30 minutes of study gas	53.8±9	52.1±9	50.3±9	50.0±10	50.1±9
Mean systolic pressure (mmHg)					
INOSG					
Baseline	70.0±13			67.9±10	
20 minutes of study gas	71.9±14			67.1±12	
INO-01/ -02					
Baseline	68.5±13	67.9±12	68.6±17	60.9±10	65.9±14
30 minutes of study gas	67.1±12	64.6±10	64.0±12	62.8±12	63.8±11
Mean diastolic pressure (mmHg)					
INOSG					
Baseline	45.8±10			47.2±10	
20 minutes of study gas	47.0±11			46.5±10	
INO-01/ -02					
Baseline	45.5±10	45.0±8	43.7±12	42.0±9	43.6±10
30 minutes of study gas	44.8±8	43.6±9	41.2±10	41.9±9	42.3±9

a. INO-01/ -02 data from NDA volume 2.18, Table T-8 to T-10.
b. INOSG data from NDA, volume 2.16, Tables T-1 to T-3. Baseline value is taken as the second baseline measurement.
c. p value for INOSG data per the sponsor, calculated using Wilcoxon Rank Sum Test of median difference of changes.'

8.1.7.2b Acute effects of I-NO on heart rate from the INOSG and INO-01/ -02 trials

Table 8.1.7.2b.1 Acute effects of I-NO on heart rate in the INOSG and INO-01/ -02 trials. Similar to above, no significant effect of I-NO on heart rate was measured.

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
Total subjects	66	41	36	63	140
Heart rate (beats per minute)[a]					
INOSG[b]					
Baseline	159±25			160±21	160±21
20 minutes of study gas	164±24			161±19	161±19
INO-01/ -02[a]					
Baseline	148±19	152±26	153±25	151±25	152±25
30 minutes of study gas	151±20	151±24	155±27	155±24	154±25

a. INO-01/ -02 data from NDA volume 2.18, Table T-11.
b. INOSG data from NDA, volume 2.16, Tables T-4. Baseline value is taken as the second baseline measurement.

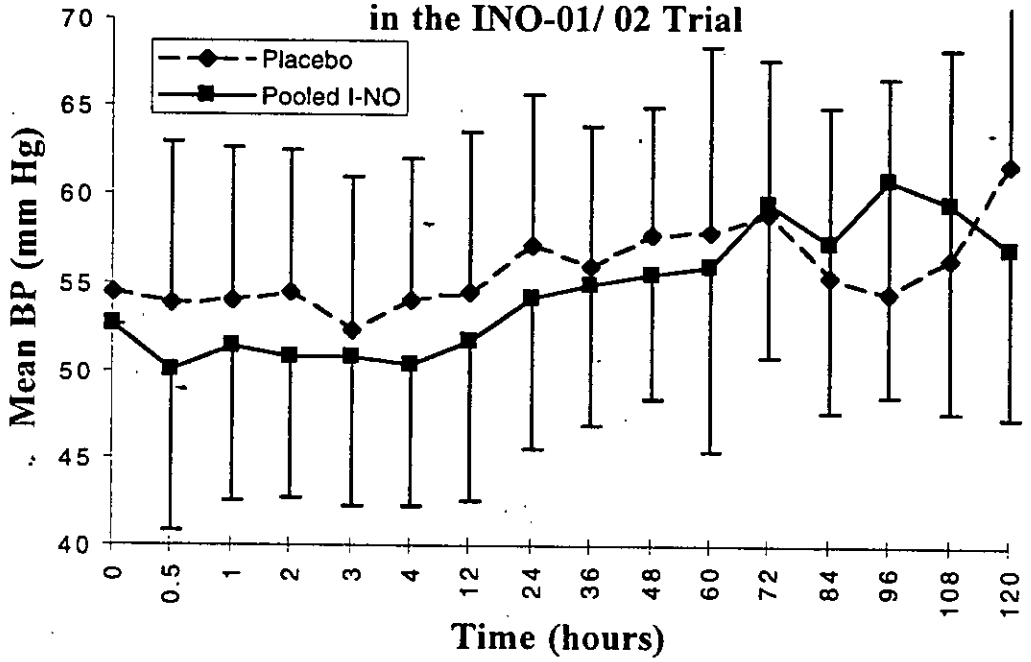
8.1.7.3 Long-term effects of I-NO on vital signs

The effect of I-NO on heart rate and BP will be examined separately.

8.1.7.3a Effect of I-NO on mean blood pressure (BP) over time

The figure below shows the mean systemic BPs from subjects exposed to control gas (N_2) and the pooled I-NO group from the INO-01/ -02 trial.

Figure 8.1.7.3.1 Mean Systemic BP vs. Time in the INO-01/ 02 Trial



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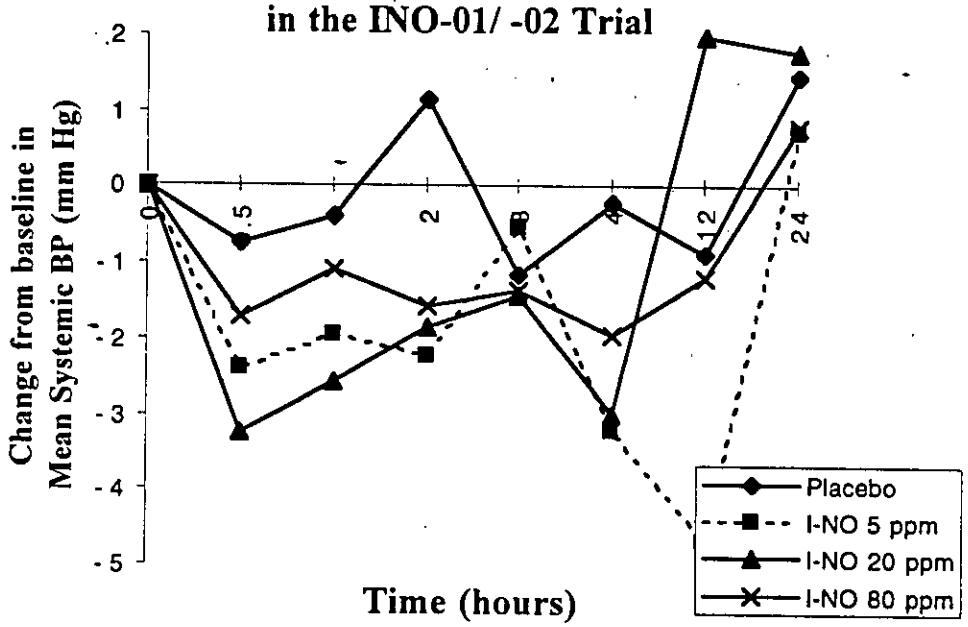
8.1.7.3a Effect of I-NO on mean blood pressure (BP) over time (cont)

The next figure looks at the effect of different doses of I-NO on BP, expressed as a change from baseline. Because of the small number of subjects for which data is available, only data up to 24 hours of study gas administration is shown. There is a suggestion that between 0 and 3 hours, I-NO lowers mean systemic BP to a greater degree than control gas (N_2). The maximal effect was seen with I-NO 20 ppm. The difference between control and the concentration with the largest effect on BP (20 ppm at 0.5 hours) was not significant (-0.73 mmHg vs. -3.22 mmHg, p = 0.30 using Student's t-test).

Figure 8.1.7.3.2 Dose-Response for I-NO on BP in the INO-01/ -02 Trial



When the effect of I-NO on mean systolic and diastolic blood pressure was examined separately a similar trend was seen. In the time period between 0.3 and 3 hours after initiation of study gas, there was a larger, non-significant decrease in the BP in the I-NO group when compared with the control group. After three hours, no discernible pattern exists.

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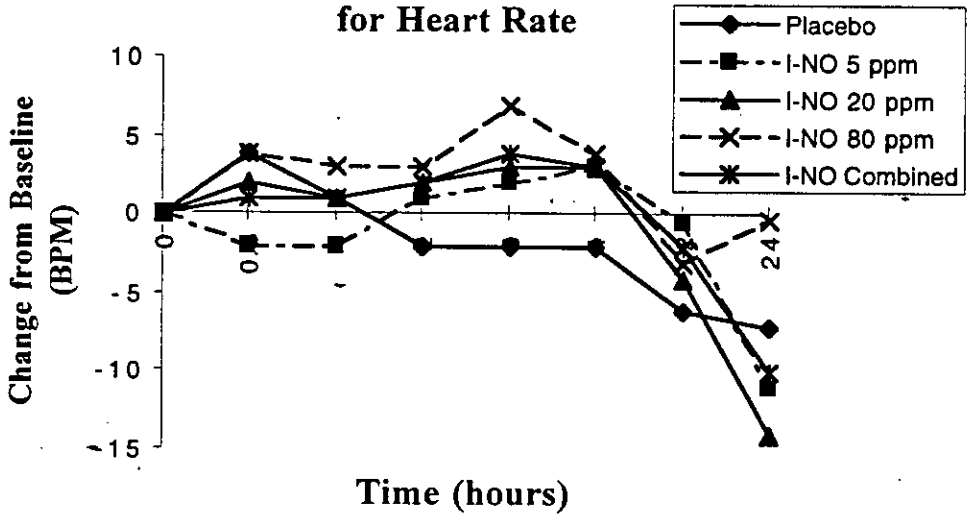
8.1.7.3b Effect of I-NO on heart rate over time

The figure below shows the change from baseline for the heart rate up to 24 hours from the INO-01/ -02 trial. No significant trend was identified.

Figure 8.1.7.3b.1 I-NO Dose-Response for Heart Rate



8.1.7.4 Vital Sign analysis focused on outliers from the NINOS, INO-01/ -02 and /-03 trials

The subjects in the table below were identified by the investigators as having an adverse event related to vital signs: arrhythmias, hypo- or hypertension, or bradycardia.

Table 8.1.7.4.1 Reported adverse events from INO-01/ -02 and INO-03 trials with reported frequency >1% or having serious clinical implications, presented by frequency within each body system for subjects receiving control gas and each of the I-NO dosage groups[a].

Body System/ adverse experience	Control Group n=41	I-NO 5 ppm n=45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	Combined I-NO n=128
Cardiovascular system	2 (5%)	5 (12%)	4 (9%)	7 (18%)	15 (12%)
Arrhythmia		2 (5%)			2 (2%)
'Heart arrest'			1 (2%)	1 (3%)	2 (2%)
Hypertension			1 (2%)	1 (3%)	2 (2%)
Hypotension		1 (2%)		1 (3%)	1 (<1%)
Bradycardia			1 (2%)	1 (3%)	2 (2%)

a. Data from NDA, volume 2.17, page 089808 to 092408, volume 2.29 page 353108 to 353308, and from individual case report forms.

Arrhythmia

Two subjects in the 5 ppm I-NO group of the INO-01/ -02 trial were reported to have had an arrhythmia as an adverse event identified by the investigators.

1) Subject 01-06002 received I-NO 5 ppm for 50 hours, and had an arrhythmia during I-NO administration lasting 5 minutes. No details of the arrhythmia are know, and no ECG is available. The subject was reported to have recovered, and was discharged without ECMO, with both reactive airways disease and seizures.

2) Subject 01-06007 received 5 ppm I-NO for 212 hours, had two episodes of arrhythmias, one during and one after I-NO therapy. The second episode was ascribed to hypotension by the investigators. No ECG or other information is available. The infant was discharged without ECMO, with no long-term adverse events identified.

8.1.7.4 Vital Sign analysis focused on outliers from the NINOS, INO-01/ -02 and /-03 trials (cont)

Bradycardia

Two subjects in the INO-01/ -02 trial, one each in the 5 and 80 ppm I-NO groups, were reported to have had bradycardia as an adverse event.

1) Subject 01-01006, a 3.7 kg white male, received treatment gas (I-NO 20 ppm) with initial improvement in oxygenation (PaO2 baseline 47, 30 minute value 202), followed by a return of hypoxemia within 36 hours despite continued I-NO. The patient continued to require maximal vasopressors for blood pressure support. His blood cultures grew group B streptococci, and the patient ultimately died after progressive hypotension, bradycardia, and a decision to withhold further support. The bradycardia was related to sepsis and withdrawal of support, rather than to I-NO therapy.

2) Subject 01-11011, a 4.2 kg white male, developed idiopathic PPHN. He was started on treatment gas (I-NO 80 ppm), with no initial response (PaO_2 was 60 at baseline, 62 after 30 minutes). There was a small, gradual increase in PaO_2 with time, and the subject received I-NO for 60 hours, at which time he developed pneumothoraces and a pneumopericardium and died. His I-NO was emergently discontinued with the development of the pneumothoraces and the cardiac arrest, which coincided with his bradycardia. He tolerated the I-NO for the 60 hours up to the time of arrest without labile blood pressures according to the case report form. While the bradycardia is temporally associated with the discontinuation of I-NO, both occurred as a consequence of cardiac arrest with pneumothoraces.

One subject in the NINOS trial had a hypertensive/bradycardic episode after temporary withdrawal from I-NO, 20 ppm.

1) Subject #12-01, a 2.8 kg male, developed pneumonia and sepsis and was treated with I-NO, 20 ppm with full response. His I-NO was disconnected for approximately 20 minutes, at which time he became hypertensive (mean arterial pressure 79 mmHg, systolic pressure 100 mmHg) and bradycardic. Labs drawn at the time showed hypokalemia and hypocalcemia. Both hypertension and bradycardia recurred when dopamine was restarted. Ultimately, the infant was continued on I-NO for another 67 hours, at which time it was removed for unresponsiveness to therapy. The investigators felt that the hypotension and bradycardia occurred as the result of an unspecified neurologic event (no neurologic adverse event is listed for the subject).

The onset of the hypertension and bradycardia are closely associated with inadvertent discontinuation of the I-NO. While no causality is clearly established for I-NO withdrawal in this subject, in the absence of other evidence for neurologic events, it is possible. Both hypocalcemia and hypokalemia can exacerbate cardiac rhythm disturbances.

Hypotension

Two subjects, one each in the 5 and 80 ppm I-NO groups, were reported to have had hypotension as an adverse event in the INO-01/ -02 trial.

1) Subject 01-04007 received I-NO 5 ppm for 13 hours, and had an episode of hypotension shortly after starting I-NO which lasted 4 hours. The subject recovered, and was discharged after ECMO with bronchopulmonary dysplasia.

2) Subject 02-11007 received I-No 80 ppm for 80 hours, and had an episode of hypotension which lasted 4 hours, starting 6 hours after the initiation of I-NO. The I-NO was continued, and the subject recovered without ECMO or other chronic adverse events.

Hypertension

Two subjects in the INO-01/ -02 trial, one each in the 20 and 80 ppm I-NO groups, were reported to have had hypertension as an adverse event.

1) Subject 01-04006, a 2.6 kg white female, was diagnosed with PPHN due to MAS, and received both ECMO and surfactant therapy. Approximately 3 weeks after being weaned from I-NO, she had hypertension (blood pressures not available) that lasted one day and did not require specific therapy. She was discharged home with bronchopulmonary dysplasia, without hypertension.

2) Subject 01-06006, a 4.1 kg black female, developed hypertension (mean arterial pressure >100 mmHg), 2 days after weaning from I-NO as a treatment failure. She later had multiple pneumothoraces, and expired. Her hypertension required specific therapy, and lasted 2 days. Hypertension was not present at the time of death.

One subject (#12-01) in the NINOS trial, discussed above in the bradycardia section, had a hypertensive/bradycardic episode after temporary withdrawal from I-NO, 20 ppm. The subject was also receiving dopamine, which can elevate the BP, although no change in dose of dopamine coincident with the increased BP was noted.

Heart Arrest

No information regarding this adverse event was available at the time of the withdrawal of the NDA.

8.1.8 ECGs

No ECG data was available for review. This lack of ECGs as part of testing of I-NO was raised with the company by the FDA in a letter sent to the sponsor on May 3, 1996. The position of the sponsor, based on the recommendations of consultant neonatologists, is that ECGs in the newborn period are used primarily to diagnose congenital heart defects, and are otherwise difficult to interpret in this population.

8.1.9 Special Studies

8.1.9.1 Special Studies: Tolerance

The potential for the development of tolerance needs to be explored. The paradigm that can be applied to I-NO and tolerance comes from the use of oral, topical, and intravenous nitrates , which also work by increasing local NO concentrations and causing vasodilation. In the case of oral and trans-dermal nitrates, tolerance is measured by at least three endpoints. Acute adminstration of oral nitrates has an anti-anginal effect, as well as improving exercise tolerance and the degree of S-T segment depression. After prolonged exposure to nitrates these effects are lost. Oral nitrates also have an acute anti-hypertensive effect, which is also lost with chronic adminstration. Of these physical manifestations of tolerance none are evaluable in the case of I-NO for neonates with hypoxic respiratory failure. The last effect, on blood pressure, cannot be assessed as there is no significant acute effect of I-NO on blood pressure in the NDA database.

One acute effect of I-NO is the improved OI and PaO_2. If there was evidence that the subjects required higher and higher amount of I-NO to maintain adequate oxygenation, this might be taken as evidence suggestive of the development of tolerance. In such a search, of course, it is important to separate the effects of disease progression, which would also cause worsening PaO_2 and OI. The protocol for the INO-01/ -02 trial, however, specified a set rate of I-NO delivery. When the subjects who remained on I-NO, at a single dose, for >12 hours, their PaO_2 tended to improve with time. If one accepts that I-NO had a part in the acute increase, this pattern suggests that there is no detectable loss of that effect over a period 12 hours to 4-5 days.

A review of the literature, where some investigators have titrated I-NO concentrations to clinical effect, revealed no subjects where the investigators reported a need to increase I-NO to maintain an initial success, which might suggest tolerance. Tachyphylaxis to the effects of I-NO has been reported(31, 51).

The sponsor has stated that they know of no data concerning the development of tolerance to I-NO.

8.1.9.2 Special Studies: Withdrawal/ 'Rebound'

The next specific adverse event associated with I-NO is an increase in pulmonary artery pressures following acute withdrawal of I-NO.

The sponsor proposed the following language regarding what it called the 'Rebound effect due to sudden withdrawal of nitric oxide:' 'Abrupt discontinuation of nitric oxide for inhalation may result in a gradual or sudden increase in pulmonary artery pressure and/or worsening of systemic oxygenation' 'Worsening of systemic oxygenation has been observed in a dose-dependent fashion when discontinuing I-NO from 1-16 ppm down to 0. In a few cases, discontinuation of nitric oxide for inhalation was associated with systemic hypotension. (Ohmeda submission, dated 9.16.97).

In the literature, the rapid withdrawal of I-NO has been associated with an acute increase in pulmonary vascular resistance and decreased arterial oxygenation (37, 44). Similar changes have been reported for intravenous nitroprusside, another vasodilator that works through the release of nitric oxide(61). This increase in pulmonary pressures results in decreased PaO_2, as the perfusion of the lung falls after pulmonary arterial pressures rise. It has been suggested that this 'rebound' increase in pulmonary arterial pressures is caused by a down-regulation of endogenous NO production due to prolonged exogenous I-NO(63, 81). Others have proposed a role for circulating Endothelin-1 and cGMP concentrations(82).

Within the NDA, the clinical effects of decreases in I-NO dose were examined in two ways. First, the effect of gradual reduction in I-NO on PaO_2 was examined in the INO-01/ -02 trial. The PaO_2 values before and after a series of 20% reductions in study gas concentration, performed during weaning of each subject, were collated and the median changes calculated for the control and I-NO groups. The table below summarizes those changes in PaO_2. It's important to note that this table does not include subjects who did not successfully wean (due to hemodynamic instability or hypoxia). There was a significant decrease in PaO_2 after weaning I-NO from 40 to 20%, and again when going from 20 to 0%.

8.1.9.2 Special Studies: Withdrawal/ 'Rebound'

Table 8.1.9.2.1 Change in baseline PaO_2 for subjects successfully weaned[a].

Study Gas %		Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
100% to 80%	Study gas conc. Median change	0 - 0 ppm -4.0	5 - 4 ppm -28.0	20-16 ppm -7.0	80 - 64 ppm -10.0
80% to 60 %	Study gas conc. Median change	0 - 0 ppm -2.0	4 - 3 ppm 2.0	16 - 12 ppm 4.0	64 - 48 ppm 2.0
60% to 40%	Study gas conc. Median change	0 - 0 ppm 7.0	3 - 2 ppm -1.0	16 - 12 ppm -3.0	48 - 32 ppm -4.0
40% to 20%	Study gas conc. Median change	0 - 0 ppm -1.0	2 - 1 ppm -14.0	12 - 8 ppm -15.0	32 - 16 ppm -7.0
20% to 0 %	Study gas conc. Median change	0 - 0 ppm 5	1 0 ppm -12.0	8 - 4 ppm -20.0	16 - 0 ppm -23.0

a. Median changes are shown. Shaded boxes differ from the control group at $p < 0.05$ using Wilcoxon rank sum test. Data from NDA, volume 2.17 Table 33, and volumes 2.24 (Appendix 16.2.2.6.3) and 2.26.

Next, the effect of abrupt withdrawal from I-NO was examined in individual subjects. In the NDA, abrupt withdrawal of I-NO was usually carried out as a result of high concentrations of methemoglobin in the I-NO 80 ppm group. Below is a table of these subjects from the INO-01/ -02 and /-03 trials, in the 80 ppm I-NO group, who had their I-NO weaned emergently (within 3-4 hours), including changes in their oxygenation parameters[b]. Subjects who had baseline hypoxia while on I-NO were not examined. This was to select those subjects who were responding to I-NO and/or were recovering from their pulmonary insult. Some, but not all, subjects emergently withdrawn had an acute decrease in PaO_2. In one case (subject 01-01005) the decrease was followed by a return to baseline within one hour, without any changes in FiO_2. In another subject, #01-3029, hypoxia required re-starting I-NO. He was later successfully weaned.

Table 8.9.1.2.2 Effect of acute withdrawal from I-NO on PaO_2[a].

Patient #	Change in I-NO	Time for withdrawal to less I-NO	Pre-withdrawal PaO_2	Post-withdrawal PaO_2
01-03029	80 to 14	1 hour	95	48
01-06006	80 to 50	4 hours	94	158
	50 to 0	4 hours	158	207
01-05005	20 to 0	1 hour	90	63
01-03003	80 to 60	0.5 hours	171	84
01-03016	80 to 60	0.5 hours	371	245
01-11004	80 to 60	0.5 hours	78	82
01-04005	80 to 40	0.5 hours	229	249
	40to 20	0.75 hours	129	76
	40 to 20	0.5 hours	162	217[c]
01-01005	80 to 20	0.5 hours	144	68
		1 hour		131[d]

a. Subjects were identified from NDA, volumes 2.26 and 2.23 and data was extracted from individual report forms.
b. Included are subjects who were tapered off of I-NO 80 ppm over 2-3 hours, where data on pre- and post-PaO2 levels are available.
c. Subject 01-04005 had two weaning attempts from 40 to 20 ppm I-NO, with widely varying results.
d. Subject 01-01005 had 30 minute and 1 hour follow-up gases after acutely decreasing I-NO from 80 to 20 ppm.

8.1.10 Abuse Potential

No data exists on the abuse potential of I-NO to the knowledge of this reviewer. 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.

8.1.11 Human Reproduction Data

There is no data on the effect of I-NO on human reproduction. Since the proposed indication is for use in neonates, the relevant issue is one of fertility of the infants after receiving I-NO. Given the short period of time since the initial studies on I-NO, no data exists to answer this question directly. A number of adult subjects have received I-NO as part of other investigations, but no data is available on any changes in reproductive capacity.

8.1.12 Overdose Experience

Human overdose data is quite limited with regards to I-NO. The sponsor has not received any information regarding adverse events which occurred to subjects in any investigational IND who received >80 ppm I-NO (personal conversation with Priya Jambekhar, Director of Regulatory Affairs for Ohmeda).

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 flowmeter 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.

8.2 Review of Systems

Section 8.2 is divided according to body systems. Within each system, a list of adverse events has been generated. The data on the association between I-NO administration and each of these adverse events will be examined in turn. In the end, a judgment regarding each relevant adverse event will be rendered: is the adverse event related to the adminstration of I-NO? In all cases, this judgment is meant only to refer to the administration of I-NO to the patient population in the NDA, and only for the doses, durations, and route of administration used in the four trials that support the NDA. To reiterate what was discussed above, the database has several limitations which make the safety assessment problematic:

1) The ability to detect unanticipated adverse events is limited by the small number of subjects with collected data for many of the adverse events.

2) Details of individual cases are limited for both the trials performed by academics (NINOS and INOSG), where only case summaries are available.

3) Extrapolation of data from the INO-01/ -02 to the entire population of infants with hypoxemic respiratory failure is difficult.

4) In the INO-01/ -02 trial, adverse events were collected by two sets of reviewers. For some adverse events, the rates detected by the two reviewers differ.

5) Some relevant adverse events had no safety data collected in any of the trials, or had too little data to make inferences concerning their association with I-NO administration.

The reader is referred to section 8.0.4 for a further discussion of these points.

Within each body system discussed below, an analysis of the adverse events will follow a set pattern. First, potential adverse events of interest will be identified from the primary NDA data (section 8.1). These will include any adverse event with >2x increased incidence in the I-NO group relative to control (see table 8.1.5.4.2). Adverse events which were followed prospectively in the trials will also be examined. Secondary data sources will also be examined for adverse events which the investigators linked to I-NO (e.g.; increased bleeding following I-NO use), as detailed in section 5.2.2.2. Finally, certain adverse events are looked for whenever new drugs are developed (e.g., hepatotoxicity; neutropenia; allergic reactions), and these will be included in the appropriate body system.

Next, the adequacy of the database to examine each adverse event will be reviewed, and the adverse events for which insufficient data exists will be identified.

For a given adverse event, the data from section 8.1 on the deaths, drop-outs and adverse events are examined, within the context of the other available data, including animal data and data from secondary sources. The first step will be to examine the adverse events identified by the individual investigators in the INO-01/ -02 and /-03 trials (these are collected in table 8.1.5.4). As discussed above, the INO-01/ -02 and /-03 trials serve as the source for all unanticipated adverse events.

Next, the adverse events for which data was collected prospectively in any or all of the four trials in the NDA will be examined (i.e., seizures, bronchopulmonary dysplasia). Deaths, drop-outs and serious adverse events will likewise be analyzed.

Then the relevant laboratory data will be scrutinized. First, the mean values for a given lab from the INO-01/ -02 trial will be examined for trends. Next, the individual subjects who developed abnormal labs will be examined, especially those who developed markedly abnormal labs.

Finally, data from secondary sources that are relevant to a particular adverse event will be cited.

The first table in this section will summarize the adverse events which occurred in the INO-01/ -02 and the INO-03 trials according to body system. Again, the NINOS and INOSG trials did not collect data in this format, so no attempt will be made to incorporate their adverse events into the table. Instead, during the discussion of the adverse events which occurred within each body system, those specific adverse events for which data exists from the NINOS and/or INOSG trials will be discussed, along with the data from the INO-01/ -02 and INO-03 trials.

8.2 Review of Systems (cont)

Table 8.2.1 Occurrence of adverse events according to body system from the INO-01/ -02 and -03 trials[a].

Body System	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Pooled I-NO
	n=41	n=41	n=36	n=37	n=114
Any adverse event	13 (32%)	19 (42%)	21 (47%)	23 (59%)	63 (49%)
Body as a whole	4 (10%)	7 (16%)	4 (9%)	4 (10%)	15 (12%)
Cardiovascular system	2 (5%)	5 (12%)	4 (9%)	7 (18%)	15 (12%)
Gastrointestinal system	0 (0%)	0 (0%)	1 (2%)	1 (3%)	2 (2%)
Endocrine system	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hemic and lymphatic system	2 (5%)	1 (2%)	5 (11%)	16 (41%)	22 (17%)
Metabolic/Nutritional system	4 (10%)	5 (11%)	7 (16%)	3 (8%)	15 (12%)
Musculoskeletal system	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (<1%)
Nervous system	4 (10%)	5 (11%)	7 (16%)	3 (8%)	15 (12%)
Respiratory system	7 (17%)	10 (22%)	11 (25%)	9 (23%)	30 (23%)
Special senses	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (<1%)
Genitourinary system	1 (2%)	3 (7%)	3 (7%)	0 (0%)	6 (5%)

a. Data from NDA, volume 2.50, page 339610 and from Table 8.1.5.4.2 above.

8.2.1 Cardiovascular System

The following potential adverse events related to the cardiovascular system were identified from the NDA, from secondary sources, or are adverse events normally explored as part of a safety review:

1) Changes in pulmonary and systemic vascular resistance.
2) Decreases in cardiac output.
3) Changes in vital signs, including arrhythmias and hypo-/ hypertension.
4) Aortic thrombosis.
5) Aortic valve vegetations.
6) Acute myocardial injury.

8.2.1.1 Adequacy of Development Program in Assessing Cardiovascular Risk for I-NO

The NDA database collected data on all adverse events in the INO-01/ INO-02 and -03 trials only, as detailed in section 8.1.7. These were defined by the sponsor as events that are not seen at baseline or, if present at baseline, have worsened in severity, and the investigators were instructed to fill out a form whenever such an adverse event occurred. This included cardiovascular adverse events, as shown in the table below. There was a higher rate of cardiovascular AEs in the I-NO group than in the control group. For overall cardiovascular adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO.

Table 8.2.1.1.1 (from table 8.1.5.4.2) Reported cardiovascular adverse events from INO-01/ -02 and INO-03 trials[a].

Body System/ adverse experience	Control Group n=41	I-NO 5 ppm n=45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	Combined I-NO n=128
Cardiovascular system	2 (5%)	5 (12%)	4 (9%)	7 (18%)	15 (12%)
Aortic Thrombosis	1 (2%)				
Arrhythmia		2 (5%)			2 (2%)
Atrial septal defect		1 (2%)			1 (<1%)
'Heart arrest'			1 (2%)	1 (3%)	2 (2%)
Hypertension			1 (2%)	1 (3%)	2 (2%)
Hemorrhage			1 (2%)	1 (3%)	2 (2%)
Cardiovascular disorder		1 (2%)			1 (<1%)
Hypotension		1 (2%)		1 (3%)	1 (<1%)
Bradycardia			1 (2%)	1 (3%)	2 (2%)
Phlebitis				1 (3%)	1 (<1%)
Vascular anomaly	1 (2%)			1 (3%)	1 (<1%)

a. Data from NDA, volume 2.17, page 089808 to 092408, volume 2.29 page 353108 to 353308, and from individual case report forms.

8.2.1.1 Adequacy of Development Program in Assessing Cardiovascular Risk for I-NO (cont)

Heart rate and blood pressure were the only cardiovascular adverse event data collected prospectively.

The collection of lab data, available from the INO-01/ -02 and /-03 trials, has been discussed previously in section 8.1.6.1 and 8.1.6.2. Two values, one at baseline and one within 12 hours of discontinuation of I-NO, are available. Follow-up for markedly abnormal labs, and labs which were identified as adverse events by the investigators, was not available. For overall cardiovascular adverse laboratory events, then, the database included 41 control subjects and 128 subjects exposed to I-NO at the time of randomization.

The NDA database collected data on heart rate, blood pressure in the INOSG and INO-01/ INO-02 trials, as detailed in section 8.1.7 above. The acute effects of administration of I-NO on these vital signs was collected for a total of 140 subjects. Long-term data (i.e., after 1 hour of I-NO administration) is available from the INO-01/ INO-02 trial, for a total of 128 subjects. Additional data for other cardiovascular events are also available in summary form from several of the published reports.

For the following adverse events, insufficient data exists to include or exclude a causal role for I-NO:

1) Changes in cardiac output

Data on cardiac output were not collected in any of the trials. This extends to follow-up echocardiograms, which were not performed or submitted as part of this NDA. No subject had 'heart failure' listed as an adverse event in the INO-01/ -02 and /-03 trials.

Overall, no conclusions concerning the effect of I-NO on cardiac output in this trial can be made.

2) Arrhythmia

Electrocardiograms (ECGs) were not collected as part of the NDA, so no data is available concerning the overall occurrence of any adverse event detected by them, including arrhythmias.

In the INO-01/ -02 trial, two subject, both in the I-NO group (5 ppm), had arrhythmia as an adverse event. These were examined in section 8.1.7.4. In neither case was there a link to the administration of I-NO.

No link between I-NO and arrhythmias has been suggested in the secondary literature, including the literature of infants with congenital heart disease and underlying rhythm disturbances.

Overall, inadequate data are available to determine if an effect of I-NO on the rate or type of arrhythmias exists.

3) Acute myocardial injury

No ECGs or creatine phosphokinase levels were obtained as part of the NDA, precluding the detection of clinical injury to the heart.

Overall, inadequate data are available to determine an association between I-NO and acute myocardial injury exists.

4) 'Heart arrest'

Two individuals are identified in the I-NO group with 'heart arrest' as an adverse event. Both infants, 01-11015 and 01-11011, died during the trial. No detailed information regarding the specifics of their 'heart arrests' is available.

Table 8.2.1.1.2 (from table 8.1.5.4.2) Specific cardiovascular adverse event from the INO-01/ -02 and /-03 trials[a].

Cardiovascular system	Control Group n=41	I-NO 5 ppm n=45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	Combined I-NO n=128
'Heart arrest'			1 (2%)	1 (3%)	2 (2%)

a. Data from NDA, volume 2.17, page 089808 to 092408, volume 2.29 page 353108 to 353308, and from individual case report forms.

Conclusion

Overall, inadequate data are available to determine an association between I-NO and 'heart arrest' exists.

8.2.1.2 Cardiovascular System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

1) Hypotension/Hypertension

In section 8.1.7.2 and 8.1.7.3, the effect of I-NO on blood pressure from the INO-01/ -02 trial were examined. Shortly after the initiation of I-NO, there appears to be a small, non-significant decrease in BP relative to control. Overall, there was no significant difference between the blood pressures in the control and I-NO groups at any time point measured.

In section 8.1.7.4, three subjects in the database who experience hypertension were examined. For two of the three infants, no association between I-NO and the hypertension can be inferred. For the third subject, (NINOS trial, #12-A01), the hypertension was temporally related to the abrupt discontinuation of I-NO, 20 ppm.

In section 8.1.7.4, two subjects in the INO-01/ -02 database who experienced hypotension after exposure to I-NO were examined (Subjects #01-04007 and #02-11007). In both cases, there was no causal link between I-NO and the lowered blood pressures identified.

Two deaths associated with hypotension occurred in the INO-01/ -02 trial, associated with documented sepsis.

1) Subject 01-10116: a 3.7 kg white male, born after 38 weeks gestation to a mother whose pregnancy was uncomplicated. The patient had Apgar scores of 8 and 9, and developed PPHN from presumed sepsis. He was started on treatment gas (I-NO 20 ppm) with initial improvement in oxygenation (PaO_2 baseline 47, 30 minute value 202), followed by a return of hypoxemia within 36 hours despite continued I-NO. The patient continued to require maximal vasopressors for blood pressure support. His blood cultures grew group B *streptococci*, and the patient ultimately died after progressive hypotension, bradycardia, and a decision to withhold further support.

2) Subject 01-11015: a 2.6 kg white male, born after a 37 week gestation, was born after an uncomplicated pregnancy. The patient's Apgars were 4 and 9, and required a chest tube placement in the delivery room for a pneumothorax. A diagnosis of PPHN from RDS was made, and the patient was started on study gas (I-NO 20 ppm) with little change in oxygenation (PaO_2 45 at baseline, 41 after 30 minutes). The patient had multiple pneumothoraces and remained hypotensive and thrombocytopenic. I-NO therapy was weaned after 140 hours. HFJV was attempted without improvement, the patient developed cystic bronchopulmonary dysplasia and *S. epidermidis* sepsis, anasarca, and ultimately died after a cardiac arrest 32 days after starting therapy.

There have been no reports of hypertensive episodes following abrupt withdrawal of I-NO in the secondary database. Increases in pulmonary vascular resistance after withdrawal of I-NO will be discussed in the respiratory system review below.

In the published literature, hypotension has rarely been described to occur following administration of I-NO, which was reversed following withdrawal of I-NO(6, 24, 31). Overall, no effect on BP has been reported.

Conclusion

No overall effect of I-NO on acute or chronic changes in blood pressure was detected in the databases. Both hypertension following the abrupt withdrawal of I-NO, and hypotension following initiation of I-NO have been reported in individual subjects. For the purposes of the safety review, both adverse events should be considered to be possibly related to I-NO.

2) Heart Rate

In sections 8.1.7.2 and 8.1.7.3, the effect of I-NO on heart rate from the INO-01/ -02 trial were examined. There was no significant effect of I-NO measured on heart rate at any time during the trial.

In section 8.1.7.4, three subjects in the database who experienced bradycardia as a specific adverse event were examined. For two of the three infants, no association between I-NO and the hypertension can be inferred. For the third subject, (NINOS trial, #12-A01), the hypertension was temporally related to the abrupt discontinuation of I-NO 20 ppm.

There were two subjects in the INO-01/ -02 trial, and one in the NINOS trial, with bradycardia recorded as a serious adverse event. Both infants in the INO-01/ -02 trial died. In both cases, the bradycardia was part of the terminal event following several days of clinical decline.

1) Subject 01-03026: a 2.8 kg Filipino female, born after 40 weeks of gestation to a mother whose pregnancy was complicated by an asymptomatic heart murmur. The patient had Apgars of 8 and 9, and developed PPHN. She was started on study gas (I-NO 80 ppm) with a dramatic, transient increase in PaO_2 (from 41 at baseline to 321 after 30 minutes to 29 after 1 hour). The patient was withdrawn from I-NO after 33 hours. The patient then received HFOV and ECMO, complicated by bleeding at the catheter site, platelet consumption, abnormal LFTs (pre-dating I-NO therapy) and worsening hypoxemia. She became bradycardic and died 12 days after starting therapy. Autopsy revealed misalignment of the pulmonary veins with alveolar capillary dysplasia.