8.2.1.2 Cardiovascular System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)

2) Heart rate (cont)

2) 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.

3) Subject 12-A01 became hypertensive and bradycardic after acute withdrawal from I-NO (20 ppm). Reinstitution of the I-NO caused a partial reversal of the bradycardia, and the infant was ultimately weaned off I-NO and was discharged.

In the published literature, an acute decrease in heart rate has also been reported following I-NO administration(5).

Conclusion

No effect of I-NO on heart rate was seen in the overall population exposed to I-NO. Following abrupt withdrawal of I-NO, some infants may be at risk for changes in heart rate, particularly bradycardia. For the purposes of this safety review, bradycardia should be considered to be possibly related to I-NO administration.

8.2.1.3 Cardiovascular System Adverse Events Considered Unlikely to be Related to I-NO

Two subjects were identified from the INO-01/-02 trial with specific adverse events related to the cardiovascular system.

1) Aortic Valve Vegetation

Patient 01-05006, developed PPHN with pneumonia and received I-NO 5 ppm for 13 hours. The infant later required ECMO and developed an aortic valve vegetation. After therapy, he was recorded to have 'improved' with regards to vegetation. On one year follow-up, no cardiovascular abnormalities were noted, had had no hospitalizations since discharge.

Conclusion

No evidence exists to link the development of the vegetation to the use of I-NO.

2) Aortic Thrombosis

Patient 01-07008, developed PPHN after meconium aspiration and sepsis, received control gas. She later developed an aortic thrombosis which was recorded as moderate in severity and improved with treatment.

Conclusion

No evidence exists to link the development of aortic thrombosis to the administration of I-NO.

8.2.2 Gastrointestinal System

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

1) Hepatotoxicity, reflected in abnormal liver function tests (LFTs).

2) Gastrointestinal bleeding.

8.2.2.1 Adequacy of Development Program in Assessing Gastrointestinal 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. This includes gastrointestinal adverse events, as shown in the table below. For overall gastrointestinal adverse events, then, the database included 41 control subjects and 128 subjects exposed to I-NO.

Table 8.2.2.1.1 (from table 8.1.5.4.2) Gastrointestinal 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
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.

Gastrointestinal bleeding was a specific adverse events looked for in the NINOS trial, where it was defined as 'frank blood per rectum or NG tube'. In this trial, data on GI bleeding before and after randomization was collected. For GI bleeding, then, data from an additional 121 controls and 114 I-NO subjects was collected (169 controls and 242 I-NO subjects total).

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 requested from the sponsor. Whenever available, this has been included in this review. For overall gastrointestinal adverse laboratory events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO.

The interpretation of I-NO effects on LFTs is complicated four things.

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

Table 8.2.2.1.2 (from table 8.1.6.2.1) Number of subjects with normal baseline LFTs from INO-01/ -02[a,c].

Laboratory	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
Alkaline Phosphatase	16/34 (47%)	12/32 (38%)	19/29 (66%)	19/29 (66%)
LDH	2/28 (7%)	5/31 (16%)	1/25 (4%)	2/29 (7%)
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%)

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).

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

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

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

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

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

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

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

8.2.2.2 Gastrointestinal System Adverse Events Possibly, Probably, or Definitely Related to I-NO

1) Hepatotoxicity, evidenced by elevated LFTs

No subjects were identified by the investigators as having an adverse events related to hepatic injury (Table 8.1.5.4.2). The following subjects had an elevation in serum bilirubin identified as an adverse event. Control and I-NO subjects had a similar incidence of bilirubinemia as an adverse event. No other LFT abnormalities were identified as adverse events in the INO-01/ -02 trial.

Table 8.2.2.2.1 Hyperbilirubinemia identified as an adverse events from INO-01/ -02 and INO-03 trials[a].

Metabolic & Nutritional	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
Bilirubinemia	2 (5%)	4 (9%)	3 (6%)	1 (3%)	8 (6%)

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

Available subject identified with increased bilirubin as an adverse event in the INO-01/ -02 and /-03 trials.

Controls

1) Subject 01-15001 received control gas for 56 hours and was discharged without ECMO, chronic lung disease or seizures.

2) Subject 02-15003 received control gas for 3 hours and was discharged without ECMO, chronic lung disease or seizures.

3) Subject 02-15004 received control gas for 8 hours. The infant had dysmorphic features and his karyotype was XXXXY. Given the poor prognosis, support was withdrawn and he ultimately died.

I-NO 5 ppm

1) Subject 01-06009 received I-NO 5 ppm for 3 hours, and recovered without ECMO. Other long-term data is missing.

2) Subject 01-14003 received I-NO 5 ppm for 24 hours, and was discharged without ECMO, chronic lung disease or seizures.

3) Subject 02-11004 received I-NO 5 ppm for 115 hours, and was discharged without ECMO, chronic lung disease or seizures.

4) Subject 02-11008 received I-NO 5 ppm for 58 hours, and was discharged without ECMO, but with reactive airways disease.

I-NO 20 ppm

1) Subject 02-07007 received I-NO 20 ppm for 2 hours, was declared a therapeutic failure, and recovered after ECMO.

2) Subject 02-15002 received I-NO 20 ppm for 6 hours, recovered and was discharged without ECMO, chronic lung disease or seizures.

3) Third subject in the 20 ppm group was not identified from the electronic datasets.

I-NO 80 ppm

1) Subject 01-01002 received I-NO 80 ppm for 8 hours, recovered, and was discharged without ECMO, but with a seizure disorder.

Drop-outs due to abnormal LFTs

There was one individual who dropped out of the INO-01/ -02 study with an elevation in LDH listed as a contributing factor.

Table 8.2.2.2.2 (from table 8.1.3.2.1.1) Subject from the INO-01/ -02 and INO-3 trials who dropped out, in part, due to an elevated LDH. No follow-up of the elevated LDH is available.

Study Group	Subject #	Adverse Event	Outcome
I-NO 5 ppm	01-01004	Acute pulmonary decompensation Elevated LDH	ECMO, HFOV, Discharged without chronic lung disease

8.2.2.2 Gastrointestinal System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)

1) Hepatotoxicity, evidenced by elevated LFTs (cont)

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

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

Lab Test[c]	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO
Alkaline Phosphatase	302.8±313 (n=40)	164±206 (n=34)	465±581 (n=38)	175±285 (n=34)	353±552 (n=36)	155±217 (n=30)	366±514 (n-34)	141±92 (n=33)
LDH	1617 ±1519 n=38	1069 ±1275 n=32	1479 ±1096 n=36	1134 ±937 n=33	3060 ±6615 n=32	1218 ±1783 n=29	1976 ±2160 n=34	1338 ±1271 n=31
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

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

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

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

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

Table 8.2.2.2.4 (from Table 8.1.6.2.2.1) Abnormal LFTs from INO-01/ -02 and INO-03[c].

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

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

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

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

8.2.2.2 Gastrointestinal System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)

1) Hepatotoxicity, evidenced by elevated LFTs (cont)

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

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

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

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

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

c. CLD chronic lung disease.

A similar trend is seen towards increased numbers of markedly abnormal LDH and total bilirubin values in the I-NO group, relative to the control group. No markedly abnormal alkaline phosphatase values were identified.

Table 8.2.2.2.6 (from table 8.1.6.2.2.1a.1) Individuals with markedly abnormal LDH and total bilirubin post-I-NO chemistry labs from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo				
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
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
I-NO 20 ppm				
01-17006	LDH	2939	3946	high
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
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
02-11007	LDH	1534	2517	high
03-59003	LDH	NA	1634	high
01-05003	Bilirubin	10.9	13.4	high
01-03005	Bilirubin	9.8	13.5	high
02-13001	Bilirubin	9.4	13.1	high

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

b. Lab tests were identified as markedly abnormal were >2X upper limits of normal on post-I-NO value.

8.2.2.2 Gastrointestinal System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)
1) Hepatotoxicity, evidenced by elevated LFTs (cont)

No follow-up on any of these abnormal labs is available. No subject is known to have chronic LFT abnormalities or chronic liver failure, as recorded at the one-year follow-up.

No subjects deaths related to liver failure, and no chronic liver abnormalities were identified from the database. One subject was known to have died with abnormal LFTs.

1). Subject 01-03025, a 4.1 kg white male, 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. Decision was made to withdraw therapy, and the infant died 1 day after weaning off I-NO.

As another index of the clinical consequences of the hyperbilirubinemia, the sponsor was asked to determine the number of subjects who received phototherapy and/or exchange transfusion for elevated bilirubin levels. This information has not been submitted to the FDA.

There was no data from the secondary sources suggesting an effect of I-NO on LFTs.

8.2.2.2 Gastrointestinal System Adverse Events Possibly, Probably, or Definitely Related to I-NO

1) Hepatotoxicity, evidenced by elevated LFTs (cont)

Conclusion

Overall, these data suggest that exposure to I-NO is possibly associated with an increase in the incidence of abnormal liver function tests, especially SGOT. No chronic damage, and no deaths due to hepatic failure were identified in the database. No firm conclusions can be drawn about the dose-relationship of this probable effect of I-NO. For the purposes of this safety review, elevated LFTs, in particular SGOT, should be considered to be possibly related to I-NO administration.

8.2.2.3 Gastrointestinal System Adverse Events Considered Unlikely to be Related to I-NO

1) Gastrointestinal bleeding

In the INO-01/ -02 and /-03 trials, one subject in the I-NO group was reported by the investigators to have had an adverse event related to gastrointestinal bleeding.

Table 8.2.2.3.1 (from table 8.1.5.4.2) Reported adverse events related to bleeding identified by investigators in the INO-01/ -02 and INO-03 trials[a].

Gastrointestinal 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
Gastrointestinal hemorrhage	0 (0%)			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.

Subjects identified as having GI bleeding as an adverse event in the INO-01/ -02 and /-03 trials.

1) Patient #02-15006 received I-NO 80 ppm group, but did not have thrombocytopenia. The gastric hemorrhage lasted 8 days, but began after the I-NO had been discontinued for 8 days. Other details are not available regarding the etiology of the GI bleed. The subject recovered completely.

In the NINOS trial, one control and 4 I-NO subjects had GI bleeding before receiving study gas. After starting study gas, one control and one I-NO subject had GI bleeding. These two individuals (11-A01 and 12-A06) both received ECMO, and both survived. No further details of their cases are available.

No data on long-term GI function or bleeding is available.

No increased risk of GI bleeding has been suggested in any trial if I-NO in the secondary database.

Conclusion

Overall, the data do not support an association between I-NO and increased GI bleeding. For the purposes of this safety review, increased GI bleeding is unlikely to be linked to I-NO administration.

8.2.3 Hemic and Lymphatic System

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

1) Elevated methemoglobin levels
2) Elevated NO_2 levels
3) Thrombocytopenia
4) Alteration in coagulation, with increased bleeding
5) Neutropenia
6) Eosinophilia

8.2.3.1 Adequacy of Development Program in Assessing Hemic and Lymphatic 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. This includes hemic and lymphatic adverse events, as shown in the table below. For overall hemic and lymphatic adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO. There was an increase in hemic & lymphatic adverse events in the I-NO group, driven the incidence of methemoglobinemia.

Table 8.2.3.1.1 (from 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
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%)

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

Specific adverse events in the hemic and lymphatic system included methemoglobin and NO_2 levels. Elevated methemoglobin were collected prospectively in the NINOS and INOSG trials, while NO_2 levels were also followed in the NINOS trial. Overall, then, 179 control and 278 I-NO subjects have methemoglobin data collected. For NO_2 levels, 151 controls and 258 I-NO subjects had data collected.

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 requested from the sponsor. Whenever available, this has been included in this review. For overall hemic and lymphatic adverse laboratory events, then, the database includes, at most, 41 control subjects and 128 subjects exposed to I-NO. The actual number of subjects with data for each lab is given when possible.

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO

1) Elevated Methemoglobin levels

The definite association between the administration of I-NO and elevated methemoglobin levels has been discussed extensively in sections 8.1.6.2.1.3 and 8.1.6.2.2.2 above. In the NINOS, INOSG and INO-01/ -02 trials there was a statistically significant association between the use of I-NO and elevated peak and mean methemoglobin levels.

In the INO-01/ -02 trial, elevated methemoglobin levels were identified as an adverse event in 13 subjects, which led to their discontinuation from I-NO (see table 8.1.3.2.1.1 above).

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%.

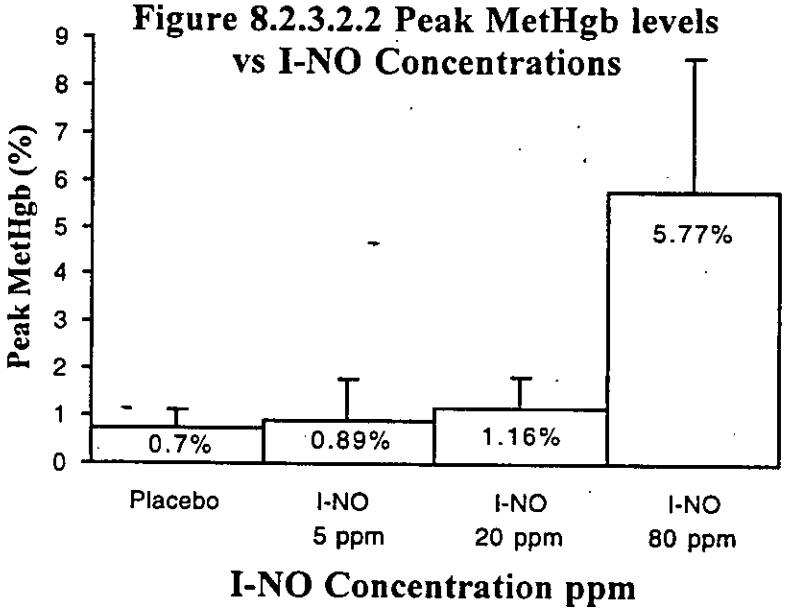
Table 8.2.3.2.1 Elevated methemoglobin levels identified as adverse events from INO-01/ -02 and INO-03 trials[a].

Hemic & Lymphatic	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
Methemoglobinemia				13 (35%)	13 (11%)

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

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)
1) Elevated Methemoglobin levels (cont)

Additionally, in the INO-01/ -02 trial, the dose-response curve was examined between I-NO and elevated methemoglobin levels. In that study, the risk of elevated methemoglobin was significantly elevated in the 80 ppm I-NO group when compared with the 20 or 5 ppm groups (data from table 8.1.6.2.1.3c.1).



All 13 subjects in the INO-01/ -02 trial who were withdrawn due to elevated methemoglobin levels were in the 80 ppm group.

This data on the association of 80 ppm I-NO with an increased risk of elevated methemoglobin levels is also in agreement with the reported effects of I-NO on methemoglobin levels in the secondary database, where elevated methemoglobin levels have been primarily reported following the use of I-NO 80 ppm(35, 57).

The clinical consequences of elevated methemoglobin clinically are less clearly demonstrated in the database. As discussed in section 8.1.6.2.2.2, examination of the 13 subjects in the INO-01/ -02 trial who were deemed treatment failure due to elevated methemoglobin levels did not demonstrate a link between them and any long-term adverse outcome, including death, need for ECMO or any marker of chronic pulmonary disease.

Conclusion

The use of I-NO is definitely linked to the development of elevated methemoglobin levels (defined as >5% in the NINOS and INOSG trials, and >7% in the INO-01/ -02 and /-03 trials). Further, there is a dose-response relationship such that subjects who received 80 ppm I-NO are at significantly higher risk for elevated methemoglobin levels. Thirty-five percent of the subjects receiving 80 ppm I-NO had elevated methemoglobin as an adverse event in the INO-01/ -02 and /-03 trials.

No clinically measurable adverse effect of methemoglobinemia was demonstrated in this trial, probably due to the short time of exposure and the small number of subjects who developed methemoglobinemia in the INO-01/ -02 trial. For the purposes of this safety review, the development of methemoglobin levels >5% should be considered to be definitely associated with I-NO administration. Further, the risks for this are significantly higher in the 80 ppm group than in the 5 or 20 ppm groups.

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)

2) Elevated NO_2 levels

The definite association between the administration of I-NO and elevated NO_2 levels has been discussed extensively in sections 8.1.6.2.1.2 and 8.1.6.2.2.1 above. In the NINOS and INO-01/ -02 trials there was a statistically significant association between the use of I-NO and elevated peak and mean methemoglobin levels.

Elevations in NO_2 levels were not identified as adverse events by the investigators in the INO-01/ -02 and /-03 trials for any subject. One subject in the INO-01/ -02 trial, #02-13001, was discontinued due to elevated NO_2 levels(see table 8.1.3.2.1.1 above).

The dose-response curve for the development of elevated NO_2 levels was also looked at in INO-01/ -02 (see section 8.1.6. 2.1.2). In the NINOS, but not in the INO-01/ -02 trials, the peak NO_2 level, measured at any time during study gas administration, was higher in the 20 ppm I-NO group when compared with placebo.

Table 8.2.3.2.3 Mean peak NO_2 after I-NO 20 ppm from the NINOS and INO-01/ -02 trials[a].

Study	Control	I-NO 20 ppm	P value
NINOS	0.1±0.3	0.8±1.2	<0.001
INO-01/ -02	0.59±0.8	0.48±.62	NS

a. Data from section 8.1.6.2.1.2 and electronic datasets, and is shown as average± standard deviation.

However, all of the subjects in the INO-01/ -02 who developed NO_2 levels >5 ppm were in the 80 ppm group. Additionally, 7 of the 11 subjects who developed NO_2 levels >3 (the definition of elevated NO_2 in the trials) were in the 80 ppm group.

Table 8.2.3.2.4 (from table 8.1.6.2.1.2c.1) Peak NO_2 levels in ppm from the INO-01/ -02 trial.

Changes in safety endpoints	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
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%)

In section 8.1.6.2.2.1, the clinical consequences of elevated NO_2 levels were considered in the individual subjects where this occurred. As noted above, no link between an elevated NO_2 level and short-term clinical toxicity could be detected, perhaps related to the small number of subjects available for examination.

Conclusion:

There is a definite link between exposure to I-NO and the development of elevated NO_2 levels. Whether subjects exposed to I-NO 20 ppm are at increased risk for elevations in NO_2 could not be determined, as the two studies who examined that group found differing conclusions.

The clinical consequences of elevated NO_2 concentrations has not been clearly established in the neonatal population, but the link to reactive airways disease and to chronic pulmonary damage (i.e., emphysema) in adults has been made in the literature (see section 5.2.2.2). The NDA database supports a possible association between I-NO and acute and chronic pulmonary damage (see respiratory section below), with a role for NO_2 in this process.

For the purposes of this review, the development of NO_2 levels >3 ppm should be considered definitely linked to the administration of I-NO. Further, there is good evidence that infants who received 80 ppm I-NO are at significantly higher risk of developing elevated NO_2 levels, compared with either 5 or 20 ppm I-NO.

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)

3) Eosinophilia

No subjects were identified by the investigators as having an adverse events related to eosinophilia (Table 8.1.5.4.2).

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

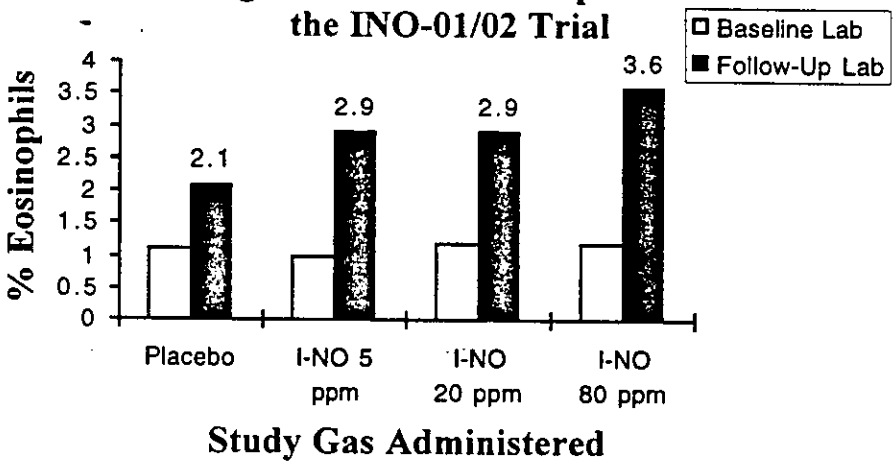
Table 8.2.3.2.5 (from table 8.1.6.2.1.1.2) Mean hematology values from INO-01/ -02[a,c].

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

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

This same data is presented as a graph below.

Figure 8.2.3.2.6 Eosinophils in the INO-01/02 Trial



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

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

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

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

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO (cont)

3) Eosinophilia (cont)

The summary of the subject who died who also had eosinophilia is below.

1. Patient 01-11012: a 3.2 kg white male, 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. No association between the death and eosinophilia is evident.

No follow-up of the eosinophilia is available for any subject.
No subjects were identified as having an adverse event or death as a consequence of eosinophilia.
No report in the secondary database links with use of I-NO with eosinophilia.

Conclusion

There is a trend towards an increased mean % of eosinophils in the database from the INO-01/ -02 trial. There is also a numerical excess of cases of eosinophilia in the I-NO group. For purposes of this review, there is a possible relationship between exposure to I-NO and eosinophilia.

4) Neutropenia

Neutropenia was not identified by an investigator as an adverse event in any subject in the INO-01/ -02 trial.

In the INO-01/ -02 trial, both control and I-NO groups demonstrated a significant decrease in WBC count. In the I-NO group, there was also a significant decrease in the % of WBC in circulation that were immature neutrophils.

Table 8.2.3.2.8 (from table 8.1.6.2.1.1.2) Mean WBC parameters from INO-01/ -02[a,c].

Lab Test	Placebo		I-NO 5 ppm		I-NO 20 ppm		I-NO 80 ppm	
	Baseline	After Study Gas	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO
WBC # (x 10^3 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 ±12.2 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

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-study gas labs differ significantly using 2-sided unpaired t test.

Control and I-NO groups in INO-01/ -02 also had similar rates of markedly low WBC counts in the INO-01/ -02 and /-03 trials.

Table 8.2.3.2.9 (from table 8.1.6.2.2.3) Abnormally low WBC counts from the INO-01/ -02 and INO-03 trials[c].

Lab Test	Control n =38	I-NO 5 ppm n = 41	I-NO 20 ppm n =32	I-NO 80 ppm n = 38	I-NO Combined
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%)

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.

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO

4) Neutropenia (cont)

There were also 3 subjects identified with markedly low post-I-NO neutrophil counts. In this analysis, both low neutrophils and low 'bands' were taken as markers for neutrophils. No subject in the database had markedly low 'WBC count.'

Table 8.2.3.2.10 (from table 8.1.6.2.2.1a.1) Individuals with markedly abnormal neutrophils labs from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo	No subjects			
I-NO 5 ppm				
02-17002	Bands	12	1	Discharged without ECMO seizures or CLD[c]
01-02002	Neutrophils	7	12	Discharged without ECMO seizures or CLD
I-NO 20 ppm				
01-07003	Bands	1	1	Discharged without ECMO seizures or CLD
I-NO 80 ppm	No subjects			

a. Data from NDA, volume 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-I-NO value.
c. CLD = Chronic Lung disease

Two subjects in the INO-01/ -02 trial, both in the I-NO group, died of infectious complications (the role of neutrophils is to fight infections). Neither of them had markedly decreased neutrophil counts.

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 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.

In the INO-01/ -02 trial, three subjects had an infectious complication listed as a serious adverse event. Two individuals in the control group had pneumonia, and one in the I-NO group had pseudomonas sepsis.

Table 8.2.3.2.10 Pneumonia as an Adverse Events from the INO-01/ -02 study[b].

Study Group	Subject #	Adverse Event	Withdrawn from Study?	Outcome	Related to Drug[a]?
Control	02-15004	Pneumonia	No	Recovered	Not Related
	02-15005	Pneumonia	No	Died	Not Related
I-NO 5 ppm	01-17003	Pseudomonas sepsis	No	Died	Not Related

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

Conclusion

There is no increased incidence of overall neutropenia in the I-NO group when compared with the control group, although in both groups there is a small decrease in the mean WBC count. There was, however, a significant decrease in the immature neutrophils in all three I-NO groups, but not in the control group (averaging approximately 40% decrease from baseline). The control group also had a non-significant decrease, and no data is available as follow-up values for the immature neutrophils.

8.2.3.2 Hemic and Lymphatic System Adverse Events Possibly, Probably, or Definitely Related to I-NO

4) Neutropenia Conclusion (cont)

While no subject was identified by the investigators as being persistently neutropenic, there were individuals in I-NO group with markedly abnormal neutrophil counts after I-NO, when compared with baseline. No such control subject was identified. There were five individuals who had an infectious complication that was serious, of which four subjects died.

For the purposes of this safety review, there is a possible association between I-NO and the development of decreased immature neutrophils. The data is not sufficient to determine if there is a clinically significant effect of this decrease.

8.2.3.3 Hemic and Lymphatic System Adverse Events Considered Unlikely to be Related to I-NO

1) Thrombocytopenia

A total of 9 subjects were identified by the investigators as having an adverse events related to thrombocytopenia (Table 8.5.4.2). Overall, there was a small, non-significant increase in the incidence of thrombocytopenia in the subjects in the I-NO group.

Table 8.2.3.3.1 Incidence of thrombocytopenia identified by an investigator as an adverse event in the INO-01/ -02 and /-03 database[a].

	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
Thrombocytopenia	1 (2%)		3 (7%)	1 (2%)	4 (3%)

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

Subjects identified as having thrombocytopenia as an adverse event in the INO-01/ -02 and /-03 trials.

1) Subject 02-07008, who received control gas, received treatment for 170 hours, and had thrombocytopenia develop and resolve during that time period. She was discharged without ECMO.

2) Subject 01-17006, who received I-NO 20 ppm, had thrombocytopenia which lasted 2 days and then recovered. The infant received gas for 8 hours at the start of those two days, was a treatment failure and was weaned off. She was discharged without ECMO, with a seizure disorder.

3) Subject 03-58001, who received 20 ppm I-NO, developed thrombocytopenia shortly after starting I-NO. She received I-NO for 19 hours, and her thrombocytopenia 'improved' after 15 hours. No further data is available on platelet counts, but she was discharge after ECMO, with no intracranial bleeding, seizures or chronic lung disease

4) Subject 03-62001, who received I-NO 20 ppm for 122 hours, developed thrombocytopenia 48 hours after starting I-NO. The thrombocytopenia resolved after 5 days, despite continued I-NO therapy. No lab data is available, and she was discharged after receiving ECMO.

5) Subject 03-59003, who received I-NO 80 ppm, developed thrombocytopenia 2 days after starting I-NO, which lasted for 2 days. He continued to receive I-NO during the thrombocytopenia, for a total of 110 hours. No follow-up lab data are available, but he was discharged with a diagnosis of bronchopulmonary dysplasia, and on supplemental O_2.

There was no trend towards a greater decrease in platelet count in the I-NO group, as seen from the average platelet count data from the INO-01/ -02 trial.

Table 8.2.3.3.2 (from table 8.1.6.2.1.1.2) Mean platelet values from INO-01/ -02[a,c].

Lab test	Placebo		5 ppm I-NO		20 ppm I-No		80 ppm I-NO	
	Baseline	After Study Gas	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO
Low platelet count[b]	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-study gas labs differ significantly using 2-sided unpaired t test.

8.2.3.3 Hemic and Lymphatic System Adverse Events Considered Unlikely to be Related to I-NO

1) Thrombocytopenia

There was also no difference in the # or % of subjects who developed abnormally low platelet counts in the control and I-NO groups in the INO-01/ -02 and /-03 trials.

Table 8.2.3.3.3 (from table 8.1.6.2.2.3) Development of thrombocytopenia from INO-01/ -02 and INO-03[c].

Low Platelet Count	Control n =38	I-NO 5 ppm n = 41	I-NO 20 ppm n =32	I-NO 80 ppm n = 38	I-NO Combined
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%)

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.

There were subjects who had a markedly abnormal number of platelets after exposure to I-NO, identified through examination of the individual laboratory records. From the table below, there was no difference in the incidence of marked abnormalities between the control and I-NO groups. No follow-up for the individual lab values is available.

Table 8.2.3.3.4 (from table 8.1.6.2.2.1a.1) Individuals with markedly abnormal post-I-NO platelet counts from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo				
01-06008	Platelets	40	57	Discharged without ECMO or bleeding
01-17002	Platelets	197	70	Discharged after ECMO without bleeding
I-NO 5 ppm				
01-03014	Platelets	171	69	Discharged without ECMO or bleeding
I-NO 20 ppm				
01-10001	Platelets	219	64	Discharged without ECMO or bleeding
01-11001	Platelets	164	10	Discharged after ECMO without bleeding
I-NO 80 ppm				
01-07004	Platelets	70	46	Discharged without ECMO or bleeding
02-07005	Platelets	166	64	Discharged after ECMO with CLD

a. Data from NDA, volume 2.25, individual patient listings

b. Lab tests were identified as markedly abnormal were <0.5X lower limit of normal on post-I-NO value.

One subject in the INO-01/ -02 trial died with thrombocytopenia.

1) 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.

Conclusion

The available data do not suggest a link between administration of I-NO and thrombocytopenia. For the purposes of this safety review, an association between I-NO and thrombocytopenia is unlikely.

8.2.3.3 Hemic and Lymphatic System Adverse Events Considered Unlikely to be Related to I-NO

2) Changes in coagulation, leading to clinically significant bleeding

As discussed in section 8.1.6.1, no markers for coagulation were submitted as part of the NDA (PT/PTT, bleeding times).

Three individuals were identified by the investigators as having an adverse event related to increased bleeding.

Table 8.2.3.3.5 (from table 8.1.5.4.2) Reported adverse events related to bleeding identified by investigators in the 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
Hemic & Lymphatic					
Anemia	1 (2%)		1 (2%)	1 (3%)	2 (2%)
Gastrointestinal system					
Gastrointestinal hemorrhage	0 (0%)	-		1 (3%)	1 (<1%)
Nervous system					
Subdural hematoma	0 (0%)		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.

These individual subjects will be discussed in the appropriate body system.

a. GI bleeding

As discussed above in section 8.2.2.3, there was no association between I-NO and increased GI bleeding detected in the database.

b. Intracranial bleeding

In the neurologic system, discussed below, the association between I-NO and intracranial bleeding is explored. The table below, from table 8.1.5.6b.1, compares the reported rates of intracranial bleeding in all of the trials. Overall, there was no increased rate of intracranial bleeding in the I-NO group compared with control.

Table 8.2.3.3.6 (from 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[b]
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.
b. p value calculated using unadjusted chi square test.

c. Pulmonary hemorrhage

In the NINOS trial, the rate of pulmonary hemorrhage was also prospectively collected. As the table below shows, there was no significant difference between the control and I-NO group rates.

Table 8.2.3.3.7 (from table 6.0.1.13.1.2) Comparison of the rate of pulmonary hemorrhage during the NINOS trial[a].

Pulmonary system	Placebo Group (n=121)	I-NO Group (n=114)
Pulmonary hemorrhage	4/110 (4%)	2/107 (2%)

a. Data shown is for adverse events which occurred after randomization..

d. Hematuria

No data was collected on hematuria for the NDA database.

8.2.3.3 Hemic and Lymphatic System Adverse Events Considered Unlikely to be Related to I-NO (cont)

2) Changes in coagulation, leading to clinically significant bleeding (cont)

e. Decreased hematocrit/ anemia

Four subjects were identified with anemia as an adverse event in the INO-01/ -02 and /-03 database.

Table 8.2.3.3.8 (from table 8.1.5.4.2) Reported anemia as an adverse event from INO-01/ -02 and INO-01/ -02-03 trials[a].

Hemic & Lymphatic 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
Anemia	1 (2%)		2 (4%)	1 (3%)	3 (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.

Subjects identified as having anemia as an adverse event in the INO-01/ -02 and /-03 trials.

1) Subject 01-06008 received control gas for 168 hours, and developed anemia during treatment, which lasted for 8 hours before resolution.

2) Subject 02-04004 received I-NO 80 ppm for <24 hours, and later developed anemia which lasted 2 days. The infant was later discharged without ECMO or other serious adverse events.

3) Subject 03-62001, who received I-NO 20 ppm for 122 hours, developed thrombocytopenia and anemia 48 hours after starting I-NO. The anemia resolved after 9 days, despite continued I-NO therapy. No follow-up lab data is available, and she was discharged after receiving ECMO.

4) Subject 03-67002 received I-NO 20 ppm, for 130 hours, during which time he developed anemia, described as iatrogenic which lasted one day before full recovery. I-NO was continued during the anemia. The infant also developed transient renal failure, and was discharged home with a seizure disorder, without having received ECMO.

In the INO-01/ -02 trial, there was a non-significant decrease in hematocrit and hemoglobin in all groups (control and I-NO). No trend towards a greater decrease in the I-NO group was seen.

Table 8.2.3.3.9 Mean hematology values from INO-01/ -02[a,c].

Lab Test	Placebo		5 ppm I-NO		20 ppm I-NO		80 ppm I-NO	
	Baseline	After study gas	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO
RBC # ($x10^6$ 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

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-study gas labs differ significantly using 2-sided unpaired t test.

A single subject was identified in the lab database with a markedly abnormal RBC count after I-NO.

1) Subject 01-05002 had his RBC count fall to markedly abnormal levels (3.8 to 2.9 $x10^6$ cells/ml). He survived after receiving I-NO 80 ppm, and both ECMO and HFOV. Further details of his clinical care are not available.

Conclusion

With the exception of increased bleeding into the bladder, which could not be assessed due to lack of data, there is no evidence that I-NO use was associated with an increase in clinically significant bleeding. For the purposes of this safety review, it is unlikely that I-NO is associated with increased clinically significant bleeding. An important inadequacy of the database is any information regarding the effect of I-NO on measures of coagulation (PT, PTT, bleeding time).

8.2.4 Metabolic and Endocrine System

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

1) Hyper- or hypo-glycemia.
2) Hyper- or hypo-natremia.
3) Hyper- or hypo-calcemia.
4) Acid-base disturbances.

8.2.4.1 Adequacy of Development Program in Assessing Metabolic and Endocrine 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. This includes metabolic and endocrine adverse events, as shown in the table below. For overall metabolic and endocrine adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO. There was no difference in the overall rate of metabolic and endocrine adverse events detected in the INO-01/ -02 and /-03 trials.

Table 8.2.4.1 (from table 8.1.5.4.2) Reported metabolic and endocrine 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
Endocrine system	1 (2%)				
adrenal insufficiency	1 (2%)				
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%)

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

No specific adverse events in the metabolic system were prospectively identified to be followed.

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 requested from the sponsor. Whenever available, this has been included in this review. For overall metabolic and endocrine adverse laboratory events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO.

No adverse events relating to the endocrine systems were identified by investigators in the INO-01/ -02 trial in the I-NO group. In the lab findings, however, several lab findings, related to the metabolic system were identified, and are summarized in the table below.

Table 8.2.4.1.1 (from Table 8.1.5.4.2) Reported metabolic and endocrine adverse events identified by investigators in the INO-01/ -02 and INO-03 trials.

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
Endocrine system	1 (2%)				
Adrenal insufficiency	1 (2%)				
Metabolic & Nutritional	4 (10%)	5 (11%)	7 (16%)	3 (8%)	15 (12%)
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%)

8.2.4.1 Adequacy of Development Program in Assessing Metabolic and Endocrine Risk for I-NO (cont)

The following adverse events cannot be assessed from the database.

1) Hyper-/Hypo-kalemia

As discussed in section 8.1.6.1, no serum electrolytes were submitted as part of the NDA. While two subjects in INO-01/ -02 and /-03 were identified as having hypokalemia (one control, one I-NO 20 ppm), this is an inadequate safety database to assess the possible effects of I-NO on serum potassium concentrations.

2) Hyper-/Hypo-natremia

As discussed in section 8.1.6.1, no serum electrolytes were submitted as part of the NDA. While two subjects in INO-01/ -02 and /-03 were identified as having hyponatremia (one control, one I-NO 80 ppm), this is an inadequate safety database to assess the possible effects of I-NO on serum sodium concentrations.

3) Acid-Base Disturbances

As discussed in section 8.1.6.1, no serum electrolytes, including serum bicarbonate, were submitted as part of the NDA. Arterial pH was assessed routinely as part of the blood gases, and the acute and chronic effects of I-NO on pH are discussed in section 6.0.3.12 of the INO-01/ -02 trial review and section 6.0.2.12.2d of the INOSG trial review.

In the INO-01/ -02 trial, no acute effect of I-NO on the acid-base balance, as measured by pH, was detected. In the INOSG trial a small increase in pH of borderline statistical significance (p=0.052) was measured following I-NO therapy for 20 minutes. No long-term effects of I-NO on pH were detected in the INO-01/ -02 trial (section 6.0.3.12), and no long-term data was collected in the INOSG trial.

No individuals were reported as having acidosis as an adverse event in the INO-01/ -02 trial, which is the only trial to collect data on acidosis as an adverse event. Given the acutely-ill nature of the infants and the poor tissue perfusion/hypoxia, this should not be taken as implying that acidosis did not occur. Rather, a large % of the subjects were receiving alkaline therapy (an average of 88% in the control group and 77% in the I-NO group of the NINOS trial), which was altered to maintain acid-base balance. Thus, while it is not possible to gauge the effects of I-NO on the acid-base status, it can be inferred that changes in the amount of alkaline therapy, and/or changes in the ventilator settings, could compensate for those undetermined changes in the trials.

4) Hyper- and hypo-calcemia

In the INO-01/ -02 and /-03 trials, three subjects were identified by investigators as having adverse events associated with changes in serum calcium levels.

Table 8.2.4.1.2 (from table 8.1.5.4.2) Reported changes in serum calcium that were identified as adverse events by investigators from INO-01/ -02 and INO-03 trials[a].

Metabolic & Nutritional 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
Hypocalcemia		1 (2%)	1 (2%)		2 (2%)
Calcium disorder			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.

Subjects identified as having hypocalcemia as an adverse event in the INO-01/ -02 and /-03 trials.

1) Subject 02-11008 received I-NO 5 ppm, developed hypocalcemia before initiating I-NO, which lasted 2 days.

2) Subject 03-59005 received I-NO 20 ppm and developed hypocalcemia which lasted 35 hours. Her baseline calcium was low (7.4). The hypocalcemia adverse event was identified at approximately the same time that her I-NO was started, but the I-NO was continued for 90 hours, during which time her hypocalcemia resolved.

3) Subject 03-67002 received I-NO 20 ppm, and had a calcium disorder in conjunction with calcification of a forehead vein secondary to infiltration of a calcium-containing solution. His serum calcium levels were normal on both baseline and follow-up.

There was no trend evident in the mean serum calcium values in the INO-01/ -02 trial.

Table 8.2.4.1.3 (from table 8.1.6.2.1.1.1) Mean clinical chemistry values from INO-01/ -02[a,b,c].

Lab Test	Placebo		5 ppm I-NO		20 ppm I-NO		80 ppm I-NO	
	Baseline	After Study gas	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO
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)

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

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

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

8.2.4.1 Adequacy of Development Program in Assessing Metabolic and Endocrine Risk for I-NO (cont)

There was, however, a trend towards a greater number of individuals in the I-NO group, especially the 80 ppm group, to develop hypocalcemia.

Table 8.2.4.1.4 (from table 8.1.6.2.2.2) Development of abnormal serum calcium values from the INO-01/ -02 and INO-03 trials.

Lab test	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
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

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.

No subjects in the INO-01/ -02 and /-03 database were identified who developed a markedly abnormal serum calcium (see table 8.1.6.2.2.1a.1) following I-NO therapy.

No effect on serum calcium has been identified in the secondary database.

Conclusion

The data in table 8.2.4.3.3 suggests a possible effect of I-NO to lower serum calcium. This is supported by the two subjects identified in the INO-01/ -02 and /-03 trials as having an adverse event related to hypocalcemia, both in the I-NO treatment groups, as well as by the increased number of newly low serum calcium in the I-NO 80 ppm group.

The total number of subjects involved, however, is quite small, and making inferences regarding adverse events occurring in 2 subjects out of 128 vs. 0 subjects out of 41 is difficult. Added to this is the difficulty interpreting serum calcium values uncorrected for the effects of alterations in serum albumin (free calcium concentrations were not measured).

For the purposes of this safety review, then, the database is insufficient to determine if an association between I-NO administration and decreased serum calcium exists.

8.2.4.2 Metabolic and Endocrine System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No adverse events in the metabolic and endocrine system fell into this category.

8.2.4.3 Metabolic and Endocrine System Adverse Events Considered Unlikely to be Related to I-NO

1) Hyper- and Hypo-glycemia

In the INO-01/ -02 and /-03 trials, two subjects were identified by investigators as having adverse events associated with changes in serum glucose levels.

Table 8.2.4.2.1 (from table 8.5.4.2) Reported adverse events related to serum glucose, from INO-01/ -02 and INO-03 trials identified by investigators[a].

Metabolic & Nutritional	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
Hypoglycemia				1 (3%)	1 (<1%)
Hyperglycemia			1 (2%)		1 (<1%)

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

8.2.4.3 Metabolic and Endocrine System Adverse Events Considered Unlikely to be Related to I-NO

1) Hyper- and Hypo-glycemia (cont)

Subjects identified as having hypoglycemia/ hyperglycemia as an adverse event in the INO-01/ -02 and /-03 trials.

1) Subject 01-15003 had hypoglycemia that started shortly after initiation of I-NO, and lasted 4 hours, resolving with specific therapy and without discontinuation of I-NO. I-NO was discontinued after 10 hours of therapy, but the subject survived without ECMO.

2) Subject 03-67002 received I-NO 20 ppm, and developed hyperglycemia which lasted 8 hours before full recovery. The hyperglycemia developed after approximately 30 hours of exposure to I-NO, and resolved without discontinuation of I-NO therapy, which continued for 136 hours . He was discharged without requiring ECMO.

There was no effect of I-NO on mean glucose concentrations in the INO-01/ -02 lab database.

Table 8.2.4.2.2 (from table 8.1.6.2.1.1.1) Mean glucose values from INO-01/ -02[a,b,c].

Lab Test	Placebo		5 ppm I-NO		20 ppm I-NO		80 ppm I-NO	
	Baseline	After Study gas	Baseline	After I-NO	Baseline	After I-NO	Baseline	After I-NO
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-study gas labs differ significantly using 2-sided unpaired t test.

At baseline, abnormalities in serum glucose were common in the INO-01/ -02 laboratory database, with 60% of subjects in groups having abnormal values at baseline (see Table 8.1.6.2.1). The majority of these abnormalities resolved during exposure to study gas. No differences in the new or worsening abnormalities were detected between groups.

Table 8.2.4.2.3 (from table 8.1.6.2.2.1) Abnormal chemistry values from the INO-01/ -02 & -03 trials[c].

Lab Test	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%)

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 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.

APPEARS THIS WAY
ON ORIGINAL

8.2.4.3 Metabolic and Endocrine System Adverse Events Considered Unlikely to be Related to I-NO
1) Hyper- and Hypo-glycemia (cont)

Finally, the number of individuals with markedly abnormal serum glucose after study gas administration is shown below. Note that both the number of markedly abnormal glucose, and the extent of abnormality is greater in the 80 ppm I-NO group than in the control. No follow-up of these laboratory values is available from the sponsor.

Table 8.2.4.2.4 (from 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				
01-17002	Glucose	71	191	Discharged without ECMO or CLD
01-07010	Glucose	140	189	Discharged after ECMO or CLD
I-NO 5 ppm				
I-NO 20 ppm				
01-03015	Glucose	135	279	Discharged without ECMO with CLD
01-11001	Glucose	190	288	Died (see below)
I-NO 80 ppm				
01-04005	Glucose	92	320	Discharged without ECMO or CLD, with seizures
02-06004	Glucose	98	217	Discharged after ECMO with no CLD
02-07003	Glucose	70	166	Discharged without ECMO or CLD
01-06003	Glucose	77	258	Discharged without ECMO (data missing)

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 lab value.

No subject died or had an identified serious adverse event as the result of changes in serum glucose. The narrative for the individual who died is below. There is no evidence available that changes in serum glucose affected his clinical course.

1) Subject 01-11001 developed idiopathic PPHN and 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. Hyperglycemia was not identified as an adverse event in this case by the investigators.

8.2.4.3 Metabolic and Endocrine System Adverse Events Considered Unlikely to be Related to I-NO
1) Hyper- and Hypo-glycemia

Conclusion

No link between exposure to I-NO and abnormalities in serum glucose is suggested by the data.

For the purposes of this review, it is unlikely that an association exists between exposure to I-NO and the development of serum glucose abnormalities.

8.2.5 Musculoskeletal

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

1) Acute or chronic muscle injury/ rhabdomyolysis

8.2.5.1 Adequacy of Development Program in Assessing Musculoskeletal Risk for I-NO

The NDA database collected data on musculo-skeletal adverse events in the INO-01/ INO-02 and -03 trials, as shown in the table below. For overall musculo-skeletal adverse events in the NDA, the database thus includes 41 control subjects and 128 subjects exposed to I-NO. One individual was identified with a pathological fracture in the I-NO 80 ppm group. The number of adverse events is too small to make any conclusions about the rate of occurrence in the control and I-NO groups overall.

Table 8.1.5.4.2 Reported musculoskeletal 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
Musculoskeletal system				1 (3%)	1 (<1%)
Pathological fracture				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.

No specific adverse events in the musculoskeletal system were prospectively identified to be followed.

In the lab database, markers for muscle injury (CPK, aldolase) were not collected.

No neurological assessments of muscle were performed routinely (e.g.; EMGs).

No potential adverse events related to the musculoskeletal system were identified from the secondary sources or are adverse events normally explored as part of a safety review.

The development program was not adequate to detect the following musculoskeletal adverse events.

1) Acute or chronic muscle injury/ rhabdomyolysis.

In the absence of CPK measurements or neurologic assessments in these sedated, paralyzed neonates, no information can be obtained concerning musculoskeletal adverse events from this database.

8.2.5.2 Musculoskeletal System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No adverse events were identified in this category.

8.2.5.3 Musculoskeletal System Adverse Events Considered Unlikely to be Related to I-NO

No adverse events were identified in this category.

8.2.6 Nervous System

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

1) Sedation.
2) Intracranial hemorrhage.
3) Brain infarct.
4) Seizures.

Additionally, given the severity of the hypoxia and hypotension that occurred during many of the subject's hospitalizations, the follow-up neurologic development of the infants in both the control and I-NO groups are of particular interest.

1) Neurologic examination 1 year after discharge.
2) Bayley scales of infant development--BSID mental scale and motor scale 1 year after discharge.
3) Incidence of cerebral palsy 1 year after discharge.

8.2.6.1 Adequacy of Development Program in assessing neurologic risk of 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. This includes neurologic adverse events, as shown in table 8.5.4.2 above. For overall neurologic adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO. No difference in the overall rate of neurologic adverse events was apparent.

Table 8.2.6.1.1 (from 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
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%)		

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

In the database, the following specific adverse events were identified prospectively in the neurologic system, and were collected prior to discharge.

Table 8.2.6.1.2 Collection of specific neurologic adverse events in the I-NO database[a].

Adverse Event	NINOS	INOSG	INO-01/ -02 and /-03
All neurologic adverse events	N	N	Y
Seizures	Y	N	Y
Brain infarct	Y	N	Y
Interventricular hemorrhage (IVH)[a]	Y	N	Y
IVH Grade I	Y	N	N
IVH Grade II	Y	N	N
IVH Grade III-IV	Y	N	N
Periventricular leukomalacia	Y	N	Y
Abnormalities on head U/S before discharge[c]	Y	N	Y
Abnormalities on CT or MRI of head[b]	Y	N	Y

a. Data from NDA volumes 2.14, 2.16, and 2.17.

b. Abnormalities specifically tabulated include: intracranial hemorrhage; interventricular hemorrhage; periventricular hemorrhage; intracranial hemorrhage; periventricular leukomalacia; extensive cytotoxic edema; subdural hematoma; ventriculomegaly; ischemia; fluid collections; and intracranial calcifications.

c. Abnormalities specifically tabulated include: brain infarct; and interventricular hemorrhage.

8.2.6.1 Adequacy of Development Program in assessing neurologic risk of I-NO (cont)

Overall, 151 control subjects and 248 I-NO subjects were followed for seizures, brain infarct, interventricular hemorrhage, and abnormalities on cranial U/S and CT. For some subjects, data was not available on all measurements (see individual sections for details).

The longer term effects of I-NO were measured at 1 year of age. Table 8.2.6.1.2 details the neurologic adverse events collected by the individual trials at this time point.

Table 8.2.6.1.2 Collection of neurologic adverse events collected at one year after discharge in the I-NO database[a].

Adverse Event	NINOS	INOSG	INO-01/ -02 and /-03
1) Neurologic examination 1 year after discharge.	N	N	Y
2) Bayley scales of infant development-- mental scale and motor scale 1 year after discharge.	N	N	Y
3) Incidence of cerebral palsy 1 year after discharge	N	N	Y

A total of 36 control and 95 I-NO subjects were available after 1 year for examination.

No laboratory data relevant to the neurologic system was identified.
No data from any EEGs or PET scans were submitted.

The following adverse events cannot be assessed from this database:

1) Sedation

Since the infants were paralyzed and sedated pharmacologically, evaluation of any sedating effect of I-NO is impossible.

2) Encephalopathy

Two infants were identified as having encephalopathy following administration of I-NO by the investigators in the INO-01/ -02 trial. Both individuals developed hypoxic encephalopathy, and one died. No association between I-NO and encephalopathy in these tw individuals is apparent. No other data on the incidence of 'encephalopathy' is available from the other trials.

Table 8.2.6.1.3 (from table 8.1.5.4.2) Encephalopathy reported as an adverse events by the investigators in the INO-01/ -02 trial[a].

Nervous 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
Encephalopathy			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.

1) 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). His examination revealed severe periventricular leukomalacia with hypoxic encephalopathy 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.

2) Subject 01-04005, a 2.9 kg Hispanic male, developed idiopathic PPHN. 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. He was diagnosed with hypoxic encephalopathy. He did not receive ECMO and was discharged without chronic lung disease.

8.2.6.2 Nervous System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No nervous system adverse events were identified in this category.

8.2.6.3 Nervous System Adverse Events Considered Unlikely to be Related to I-NO

1) Intracranial hemorrhage

One I-NO subjects had intracranial bleeding that was identified by investigators as an adverse event in the INO-017 -02 and /-03 trials.

Table 8.2.6.2.1 (from table 8.1.5.4.2) Reported intracranial bleeding events identified as adverse events by investigators in the INO-01/ -02 and INO-03 trials[a].

Nervous 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
Subdural hematoma			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.

One subject was identified as having a subdural hematoma as an adverse event in the INO-01/–02 and /-03 trials.

1) Subject 02-04007 received I-NO, 20 ppm, for one day, then developed a subdural hematoma 17 days later. The infant received ECMO and was discharged home, with the status of the subdural hematoma recorded as 'continuing.' At the one-year follow-up, mild hearing deficit was noted, but no neurologic abnormalities. No association of the subdural hematoma to I-NO administration is evident.

The overall rate of intracranial hemorrhage was collected in the NINOS and INO-01/ -02 and /-03 trials, and, as shown below, there was no significant difference in the rate between control and I-NO groups.

Table 8.2.6.2.2 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. Data from individual trial results, sections 6.0.1 to 6.0.4.

Similar analyses can be performed from the INO-01/ -02 trial, pooling all intracranial bleeding events as 'intracranial hemorrhage'[a]. This data comes from the smaller pool of subjects who had MRI or CAT scans before discharge. Since these scans were not specified by protocol, interpretation of these small numbers of subjects is difficult, although there is no suggestion of increased intracranial bleeding in the I-NO group.

Table 8.2.6.2.3 Reported intracranial hemorrhages detected by MRI or CAT scan in the INO-01/ -02 trial[a].

	Control Group n=18	I-NO 5 ppm n=15	I-NO 20 ppm n=19	I-NO 80 ppm n=11	Combined I-NO n=45
Normal Scan	9 (50%)13	13 (87%)	11 (58%)	7 (64%)	31 (69%)
Intracranial bleeding[a]	3 (17%)	1 (7%)	2 (10%)	1 (9%)	4 (9%)

a. Events which are counted as intracranial bleeding include: interventricular hemorrhage; small frontal intracranial hemorrhage; periventricular hemorrhage; subdural hematoma; posterior fossa hemorrhage; and parietal hemorrhage.

Two infants in the I-NO group died with neurologic complications.

1) 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.

2) 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.

8.2.6.3 Nervous System Adverse Events Considered Unlikely to be Related to I-NO

1) Intracranial hemorrhage (cont)

In one study in the secondary database, survival without intracranial hemorrhage or asthma was reduced in the I-NO group relative to the an historical control group (36). In another trial, 2 of 8 infants died of intraventricular hemorrhage >24 hours after discontinuing I-NO (43). In the data submitted by Dr. Wessel as part of his IND(21), there was a decreased risk of seizures or intracranial hemorrhage in the I-NO group (8/23 in control, 4/29 in I-NO, see section 5.2.1). Information regarding relative contribution of seizures and intracranial hemorrhage not available.

Conclusion

No evidence in the NDA database suggests an association between I-NO administration and increased intracranial bleeding. There is a single published report of 2/8 subjects who had interventricular bleeding after exposure to I-NO. For purposes of this review, no association between short-term I-NO administration and intracranial bleeding was detected.

2) Seizures

Two control subjects and 9 I-NO subjects were identified by investigators in the INO-01/ -02 and /-03 trials as having 'convulsion' as an adverse event. None of the subjects with adequate data had their seizures start after initiation of I-NO therapy and disappear after stopping I-NO.

Table 8.2.6.2.4 (from table 8.1.5.4.2) Reported convulsions identified as adverse events by investigators in the INO-01/ -02 and INO-03 trials[a].

Nervous 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
Convulsion	2 (5%)	2 (4%)	5 (11%)	2 (5%)	9 (7%)

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

The following subjects had seizures reported as adverse events in the electronic database.

Control

1) Subject 01-04001 had a single seizure, and was discharged without ECMO, seizure medications or chronic lung disease (CLD).

2) Subject 02-04005 had a single seizure lasting 2 minutes, recovered and was discharged without ECMO, seizure medications or CLD.

I-NO 5 ppm

1) Subject 01-06002 had a single seizure, recovered and was discharged without ECMO with CLD. Other data about status at discharge is missing.

2) Subject 02-14006 had a single seizure lasting <2 minutes, recovered and was discharged home without ECMO or CLD.

I-NO 20 ppm

1) Subject 01-06004 had a single seizure lasting 3 minutes, recovered and was discharged without ECMO or CLD.

2) Subject 01-09004 developed an ongoing seizure disorder, received ECMO, and suffered a brain infarct. He was discharged home with ongoing seizure disorder.

3) Subject 01-03025 had seizures which were categorized as severe, before starting I-NO, associated with encephalopathy. Ultimately the subject died after evaluation of the infant revealed severe periventricular leukomalacia and a burst pattern on EEG. The infant also had persistent renal failure.

4) Subject 03-62001 received I-NO 20 ppm, and had seizures associated with a left temporal cortical hemorrhage and cerebral infarct. She received ECMO.

5) Subject 03-67002 received I-NO and had seizures associated with 'Focal Cerebral Dysfunction' which lasted 10 minutes. He recovered without ECMO.

I-NO 80 ppm

1) Subject 01-01002 had a single seizure, recovered and was discharged after receiving ECMO on no seizure medications.

2) Subject 01-05003 had 'questionable seizure' that lasted 6 hours and started before I-NO was administered. The subject recovered and was discharged without ECMO.

8.2.6.3 Nervous System Adverse Events Considered Unlikely to be Related to I-NO

2) Seizures

In the NINOS and INO-01/ -02 and /-03 trials, the incidence of seizures was a safety event which was monitored prospectively. The table below summarizes the reported rates of seizures in this database. There is no clear difference in the rate of seizures in the two groups.

Table 8.2.6.2.5 Reported seizures from NINOS, INOSG, INO-01/ -02 and INO-03[a].

Trial	Control	I-NO	p value
NINOS	20/121 (17%)	13/114 (11%)	0.26
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. Data from NDA volume 2.14 Table 31, INO-01/ -02 electronic datasets, and volume 2.31.

One infant had refractory seizures and died in the I-NO group. The seizures started before I-NO was initiated.

1) 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.

Seizures were also reported as serious adverse events in two subjects in the INO-01/ -02 trial.

Table 8.2.6.2.6 (from table 8.1.2.2.1) Seizures identified as serious adverse events from the INO-01/ -02 study[b].

Study Group	Subject #	Adverse Event	Withdrawn from Study?	Outcome	Related to Drug[a]?
Control	01-04001	Seizure	No	Recovered	Not Related
I-NO 20 ppm	01-03025	Renal Failure	No	Sequelae	Not Related
		Encephalopathy	No	Died	Not Related
		Seizures	No	Sequelae	Not Related
		Pneumothorax	No	Sequelae	Not Related

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

No association between I-NO administration and seizures has been reported in the literature. In the data submitted by as part of his IND [in press (21)], there was a decreased risk of seizures or intracranial hemorrhage in the I-NO group (8/23 in control, 4/29 in I-NO, see section 5.2.1). Information regarding relative contribution of seizures and intracranial hemorrhage not available.

Conclusion

There is no evidence suggesting a link between I-NO administration and the incidence of seizures. For the purposes of this review, seizures are unlikely to be related to short-term I-NO administration.

3) Brain Infarct

Two subjects were identified by investigators in the INO-01/ -02 and /-03 trials as having 'cerebral infarct' as an adverse event, one in the control group and one in the I-NO 20 ppm group.

Table 8.2.6.2.7 (from table 8.1.5.4.2) Reported neurologic adverse events identified by investigators in the INO-01/ -02 and INO-03 trials[a].

Nervous 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
Cerebral infarct	1 (2%)		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.

8.2.6.3 Nervous System Adverse Events Considered Unlikely to be Related to I-NO (cont)

3) Brain Infarct (cont)

One subject was identified as having a cerebral infarct as an adverse event in the INO-01/ -02 and /-03 trials.

1) Subject 02-15004 received control gas for 4 hours, and had a small cerebral infarct detected on ultrasound.

The subject who received I-NO and had a cerebral infarct is not identified in the electronic database and was not available from the sponsor at the time the NDA was withdrawn.

Brain infarct was also identified as a prospectively collected adverse event in the NINOS and INO-01/ -02 and /-03 trials.

Table 8.2.6.2.8 Incidence of 'brain infarct' in the NINOS trial and INO-01/ -02 and /-03 trials[a].

Study	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
NINOS	4/82 (5%)		7/77 (9%)		
INO-01/ -02 and /-03	2/39 (5%)	0/34 (0%)	4/33 (12%)	1/31 (3%)	5/150 (3%)

a. Data shown is for adverse events which occurred after randomization, and is shown for all subjects with available data.

No information from the secondary database suggests a relationship between I-NO administration and the rate of cerebral infarction.

Conclusion

While the incidence rate of 'brain infarct' was more than twice the control rate in the 20 ppm I-NO in the INO-01/ -02 and /-03 trials, this represents only 4 individuals. Furthermore, the overall rate, and the rate for the I-NO 80 ppm groups are not different from the control rate. For the purposes of this review, cerebral infarction is unlikely to be related to the short-term administration of I-NO.

4) Long-term neurologic development

Long-term neurologic development was examined in the INO-01/ -02 and NINOS trials. The only data that is available for this review, however, are from the INO-01/ -02 trial, and were discussed in sections 6.0.3.13.3 and 8.1.5.6f. Categories of neurologic development that were examined after 1 year include: mental development; psychomotor development; neurologic abnormalities; and cerebral palsy.

Of the long-term adverse events prospectively followed, none show any increased rate of occurrence in the infants who had received I-NO.

Mental Development

Table 8.2.6.2.9 (from table 6.0.3.13.3.3) Mental development at 1 year of age for infants with known follow-up in INO-01/ -02[a,b].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
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

b. Data from NDA Amendment submitted August 28, 1997, Volume 6.1.

8.2.6.3 Nervous System Adverse Events Considered Unlikely to be Related to I-NO (cont)

Psychomotor Development

Table 8.2.6.2.10 (from table 6.0.3.13.3.4) Psychomotor development at 1 year of age for infants with known follow-up in INO-01/ -02[a,b].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
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 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

b. Data from NDA Amendment submitted August 28, 1997, Volume 6.1.

Audiology Testing

Table 8.2.6.2.6.11 (from table 6.0.3.13.3.5) Results of audiology testing at 1 year of age for infants with known follow-up in INO-01/ -02[a,b].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
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

b. Data from NDA Amendment submitted August 28, 1997, Volume 6.1.

Neurologic Testing

Table 8.2.6.2.6.12 (from table 6.0.3.13.3.6) Results of neurologic examination at 1 year of age for infants with known follow-up in INO-01/ -02[a,b].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
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.
b. Data from NDA Amendment submitted August 28, 1997, Volume 6.1.

8.2.6.3 Nervous System Adverse Events Considered Unlikely to be Related to I-NO (cont)

Incidence of Cerebral Palsy

Table 8.2.6.2.6.13 Incidence of cerebral palsy at 1 year of age for infants with known follow-up in INO-01/-02[a,b].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
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 if cerebral palsy was present or not.
b. Data from NDA Amendment submitted August 28, 1997, Volume 6.1.

Conclusion

There is no data in the long-term safety database from INO-01/-02 that I-NO administration is associated with an increased rate of any of the neurologic abnormalities tested for during the follow-up visit. For the purposes of this safety review, I-NO is unlikely to be associated an increased rate of any of the listed long-term neurologic events.

8.2.7 Respiratory system

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

1) Air leak syndrome (pneumothorax, pneumomediastinum, interstitial emphysema, pneumopericardium).
2) Chronic pulmonary injury.
3) Decreased surfactant production.
4) Decrease in PaO_2 and increase in pulmonary hypertension following withdrawal from I-NO.
5) Increased bronchial reactivity/ reactive airways disease.
6) CO_2 retention.
7) Need for supplemental O_2 at time of discharge.
8) Decreased PaO_2 as a result of decreased FiO_2 during I-NO administration.

8.2.7.1 Adequacy of Development Program in Assessing Respiratory 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. This includes the respiratory system adverse events, as shown in the table below. For overall respiratory adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO. There was a small increase, both numerically and percentage-wise, in the rate of overall respiratory adverse events.

Table 8.2.7.1.1 (from table 8.1.5.4.2) Reported respiratory adverse events from INO-01/ -02 and INO-03[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
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%)				

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

The database for assessing the respiratory risks of exposure to I-NO include the following components:

1) Acute data on the effects of I-NO on PaO_2 comes from the INO-01/ -02 and /-03, NINOS and INOSG trials. This data was collected at baseline and again after either 20 or 30 minutes of study gas. This represents a total number of exposed subject of 179 control and 278 I-NO subjects (from Table 8.0.3.1).

2) Acute data on the effects of I-NO on pCO_2, and pH from the INO-01/ -02, and INOSG trials (it was not collected in the NINOS trial). This data was collected at baseline and again after either 20 or 30 minutes of study gas. This represents a total number of exposed subject of 69 control and 141 I-NO subjects (from Table 8.0.3.1).

3) Chronic data on the effects of I-NO on PaO_2, pCO_2, and pH from the INO-01/ -02 trial. This data was collected routinely during study gas administration for all subjects in the two trials. No data were collected or submitted after discontinuation of the study gas. This represents a total of 41 control and 114 I-NO subjects.

4) Chronic data, up to the time of discharge, was also collected in all 4 trials regarding the incidence of specific pulmonary adverse events. These are summarized below.

8.2.7.1 Adequacy of Development Program in Assessing Respiratory Risk for I-NO (cont)

Table 8.2.7.1.2 Collection of respiratory adverse events in the I-NO database[a].

Adverse Event	NINOS	INOSG	INO-01/ -02 and /-03
Acute changes in oxygenation parameter	Y[d]	Y	Y
Chronic changes in oxygenation parameters	N	N	Y
All adverse events identified by investigators	N	N	Y
Air leak syndrome	Y	N	Y
Pulmonary hemorrhage	Y	N	N
Chronic lung disease	Y	Y	N[b]
Reactive airways disease	N[c]	N	Y
Requiring O_2 after 28 days	N[c]	Y	Y
Bronchopulmonary dysplasia	N	N	Y

a. Data from NDA volumes 2.14, 2.16, and 2.17.

b. In the INO-01/ -02 trial, bronchopulmonary dysplasia, rather than chronic lung disease, was a specific adverse event, defined as O_2 >21% required at 28 days of age with abnormal chest x-ray ray or the use of bronchodilators.

c. In the NINOS trial, chronic lung disease was a specific adverse event, defined as defined as O_2 >21% required at 28±7 days of age with abnormal chest x-ray.

d. NINOS did not collect PCO_2 data at any time point.

5) Finally, as part of the long-term follow-up, two sets of information potentially related to the respiratory system were collected. First, as part of the standard examination Review of Systems, the subject and caregivers were asked about 'Respiratory' problems, including: home O_2 therapy; asthma; bronchiolitis; bronchitis; pneumonia; upper respiratory infection with severe cough; and smoking in household. Examinations were performed on 36 controls and 95 I-NO subjects. Second, caregivers were asked about hospitalizations since birth, and details were noted in the record.

Progressive hypoxemia and/or pneumothoraces were contributory in the deaths of several of the infants in the INO-01/ -02 trial. These children, along with their treatment and details of their deaths, are summarized in the table below. Details of their deaths can be found in section 8.1.1.1 above. Refractory hypoxemia despite ECMO and I-NO was a common thread in the observed deaths in the INO-01/ -02 trial.

Table 8.2.7.1.3 Deaths with respiratory adverse events in the INO-01/ -02 trial[a].

Subject	Duration of study gas (hrs)	Air Leak Syndrome?[b]	Refractory Hypoxemia	Support Withdrawn?	Notes
Control					
02-15005	8	No	Yes	Yes	
I-NO 5 ppm					
01-11012	10	Yes	Yes	Yes	
01-17003	25	No	Yes	No	
I-NO 20 ppm					
01-11005	56	Yes	Yes	No	
I-NO 80 ppm					
01-03026	33	No	Yes	No	Alveolar-capillary dysplasia
01-06006	144	Yes	Yes	No	Persistent methemoglobinemia
01-11011	60	Yes	No	No	Pneumopericardium

a. Data from section 8.1.1.1 and electronic datasets.

The database is insufficient to assess the relationship between I-NO and the following adverse event:

1) Surfactant dysfunction related to I-NO production

Evaluation of any effect of I-NO on surfactant production is complicated by the fact that a high % of subjects in the NINOS trial received surfactant (54% of the control and 38% of the I-NO group), while no subject in the INO-01/ -02 could be using it. The use of surfactant did not alter the % of subjects who responded to I-NO in the NINOS trial (see table 6.0.1.12.3b.1 above).

8.2.7.1 Adequacy of Development Program in Assessing Respiratory Risk for I-NO (cont)

In the INO-01/ -02 and /-03 trial, two subjects were identified as having 'secondary surfactant deficiency.'

1) Subject #02-15003, who received I-NO 80 ppm, had 'secondary surfactant deficiency' that lasted 10.5 hours, with recovery after surfactant therapy, and was discharged without evidence for long-term respiratory dysfunction.

2) Subject #02-15004, who received control gas, had 'secondary surfactant deficiency' that lasted 2.25 days, with recovery after surfactant therapy, and was discharged without evidence for long-term respiratory dysfunction.

In the secondary database, one author suggested an adverse effect of I-NO on surfactant production, which was improved with ECMO(50).

Conclusion

While there is nothing in the database which suggests an effect of I-NO on surfactant, the database is inadequate to assess the potential effects of I-NO on surfactant production and/or function.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

1) Decreased PaO_2 and increased pulmonary vascular resistance (PVR) following withdrawal from I-NO

The phenomenon of increased PVR following withdrawal of I-NO was first raised in the literature in subjects undergoing repair of congenital cardiac lesions. In the NDA, the change in PaO_2 following decreases in the amount of administered I-NO was specifically examined in the INO-01/ -02 trial, and the results detailed in section 8.1.9.2. There was a significant decrease in PaO_2 following weaning from I-NO, particularly during weaning from 40 to 20% and complete withdrawal from I-NO. Some subjects required multiple weaning attempts before their I-NO concentrations could be reduced.

Additionally, subjects who were acutely withdrawn from I-NO frequently, but not uniformly, had decreases in PaO_2. In at least one subject (01-01005) the decrease in PaO_2 was transient, lasting less than one hour. In another subject (01-03029), hypoxia required re-starting I-NO. He was later successfully weaned.

No data exists in the NDA concerning the effects of withdrawal on pulmonary vascular resistance or pulmonary arterial pressure. Increases in PVR are the presumed mechanism for the hypoxia observed, in agreement with the data from the secondary database. It has been suggested in the literature that this reflects a decreased endogenous NO production following long-term I-NO administration ('down-regulation') (63).

Conclusion:

There is a definite association between withdrawal of I-NO and increased hypoxia. This occurs both during routine weaning and during acute withdrawal of I-NO. In most cases, the hypoxia can be managed by increasing the FIO_2 and/or resolves without specific treatment, but can require re-institution of I-NO. For the purposes of this review, there is a definite association between withdrawal from I-NO and a transient decrease in PaO_2.

2). Reactive Airways Disease

'Asthma' was identified as an adverse event in the INO-01/ -02 trial. Exactly what was meant by this clinically is not known (apparently true 'asthma' is quite rare in the neonatal population). Per the definition of an adverse event, clinical signs of bronchospasm developed, or worsened, after exposure to study gas.

The table below list the rate of 'asthma' identified by the investigators as and adverse event in the INO-01/ -02 and /-03 trials. There was a greater incidence of asthma in subjects exposed to I-NO (in fact, asthma was not reported in the control group).

Table 8.2.7.2.1 (from table 8.1.5.4.2). Incidence of 'asthma' identified as adverse events in INO-01/ -02 and /-03 trials[a].

Adverse Event	Control n=41	I-NO 5 ppm n= 45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	I-NO Combined n=128
Asthma	0 (0%)	2 (4%)	2 (4%)	1 (2%)	5 (4%)

a. Data from individual patient listings, NDA volumes 2.26 and 2.31, appendix 16.2.2.21, completed at the end of hospitalization for all surviving subjects. Data shown as % of all subjects with data.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
2). Reactive Airways Disease (cont)

There was also a small, non-significant, increase in the rate of 'Reactive Airways Disease', collected prospectively in the INO-01/ -02 and /-03 trials. NINOS and INOSG did not collect data on the incidence of asthma or reactive airways disease.

Table 8.2.7.2.2 Incidence of 'Reactive Airway Disease from the INO-01/ -02 trial[a].

Adverse Event	Control n=40	I-NO 5 ppm n= 42	I-NO 20 ppm n=38	I-NO 80 ppm n=39	I-NO Combined n=128
Reactive Airways Disease	1 (2.5%)	3 (7%)	1 (3%)	1 (3%)	5 (4%)

a. Data from individual patient listings, NDA volumes 2.26 and 2.31, appendix 16.2.2.21, completed at the end of hospitalization for all surviving subjects. Data shown as % of all subjects with data.

At the reviewers request, the sponsor also submitted case report forms for the subjects who had were identified at discharge as having reactive airways disease (or asthma). The discrepancy between the number of subjects with asthma identified by the sponsor (7) and the number in table (5) has not been resolved. Note that two of the subjects received HFOV/HFJV, which has been identified as an independent risk for pulmonary injury. Also note that, with the exception of subjects 1 and 4, the subjects were all exposed to I-NO for ≥50 hours.

First, the subjects with asthma in the INO-01/ -02 trial are identified below, along with their underlying disease state and clinical outcome.

1) Subject 02-14007, developed PPHN due to RDS with RDS, received I-NO 20 ppm for 3 hours, and developed asthma 16 days after discontinuing the I-NO. The infant did not receive ECMO but received HFOV, and was discharged with BDP and reactive airways disease. His asthma was still present at the one-year follow-up. He required supplementary O_2 for the first month after discharge.

2) Subject 02-11008, developed PPHN due to MAS, received I-NO 5 ppm for 57 hours, and developed reactive airways disease 16 days after discontinuing I-NO. He was discharged without ECMO, HFOV or HFJV, with reactive airways disease and bronchopulmonary dysplasia. After one year, he was reported to have bronchiolitis, but no asthma.

3) Subject 02-17002, an Hispanic male, developed PPHN after MAS. He received I-NO 5 ppm for 129 hours, did not receive ECMO, HFOV or HFJV, and was discharged home without chronic airways disease or supplemental O_2. He had bronchitis and a history of pneumonia at the one-year follow-up.

4) Subject 01-11007, a white female, developed PPHN after MAS. She received I-NO 5 ppm for 9 hours, received HFOV and ECMO, and was discharged home with seizures, reactive airways disease and bronchopulmonary dysplasia.

Three subjects with asthma was identified in the INO-03 trial.

5) 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 for reactive airways disease. Long-term follow-up is not available.

6) Subject 03-59003, a black male, developed PPHN after MAS. Asthma was noted 10 days after starting I-NO. He received I-NO 80 ppm for 75 hours, did not receive ECMO, HFOV or HFJV, and was discharged home with reactive airways disease. Of note, the subject also had elevated methemoglobin levels (5.7 after 64 hours) and elevated NO_2 levels (>3.0 ppm for >3 hours, peak 3.3 ppm after 11.6 hours). No one-year follow-up data is available.

7) Subject 03-67001, a black male, developed PPHN after MAS. Asthma was noted 14 days after ending I-NO 80 ppm, which he received for 75 hours. The subject did not receive ECMO, HFOV or HFJV, and was discharged home with reactive airways disease. 6) Subject 03-67001, received I-NO 20 ppm for 132 hours, did not receive ECMO, had BPD and reactive airways disease at discharge. Neither NO_2 levels nor methemoglobin levels were elevated at any time. No one-year follow-up data is available.

The secondary database also includes a report of reactive airways disease following exposure to I-NO (42). In this study 3/10 subjects who received I-NO 8-80 ppm for more than one week developed bronchopulmonary dysplasia, which the authors speculated might be related to the administration of I-NO.

The potential mechanism for such an effect of I-NO on the airways is not established, but several possibilities exist. First, I-NO may be a direct irritant. This is not supported by the observation that several of the infants developed 'asthma' >10 days after stopping the I-NO. Second, I-NO may impair normal lung function, either through inhibiting the production of normal proteins (surfactant), or by damaging long-lived proteins, leading to progressive pulmonary injury. The latter mechanism could be mediated by the production of NO_2 from I-NO and O_2 in the lungs of infants. Such an effect might well take several days to be manifest clinically.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
2). Reactive Airways Disease (cont)

Conclusion

The data suggests that there is a possible association between I-NO administration and reactive airways disease for up to 28 days after exposure to I-NO. For the purposes of this safety review, a possible link exists between exposure to I-NO and the development of reactive airways disease. There is not sufficient data to analyze a dose-effect for this adverse event.

3) Air Leak Syndrome (ALS)

This syndrome includes pneumothorax, pneumomediastinum, pneumopericardium, and interstitial/subcutaneous emphysema.

First, the rate of ALS was collected in both the NINOS and INO-01/ -02 and /-03 trials as prospectively-identified adverse events.

There was no difference in the incidence of 'Air leak syndrome' after 28 days in either the INO-01/ -02 and /-03 or NINOS trials. In the NINOS trial, however, there was a numerical and percentage increase in the subjects with ALS during I-NO administration. The rate of pneumothorax, extracted from the list of subjects in the INO-01/ -02 with 'Air Leak Syndrome' was also not different between the two groups.

Table 8.2.7.2. 3 Incidence of air leak syndrome specifically collected as a category in the NINOS, INO-01/ -02 and /-03 trials.

Adverse Event	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
INO-01/ -02 Trial					
Air leak syndrome	16/41 (39%)	13/45 (29%)	20/44 (45%)	14/59 (36%)	47/128 (37%)
Pneumothorax	12/41 (29%)	7/45 (16%)	10/44 (23%)	9/59 (23%)	26/128 (20%)
NINOS Trial					
Airleak syndrome during and after study gas[b]	19/121 (16%)		21/110 (19%)		
Air leak syndrome during study gas[c]	7/121 (6%)		12/110 (11%)		

a. Data for INO-01/ -02 and /-03 from individual patient listings, NDA volume 2.17, table 53, and volumes 2.26 and 2.31, appendix 16.2.2.20, completed at the end of hospitalization for all surviving subjects. Data for NINOS from section 6.0.1.13.1.

b. This column includes all subjects who had met criteria for 'Air leak syndrome' up to time of discharge, and is derived from Table 6.0.1.13.1.2. The subjects who had air leaks before randomization are not included in any statistic in this table.

c. Data from table 6.0.1.13.1.2.

However, in the INO-01/ -02 trial, there was a greater incidence of pneumothoraces identified as adverse events in subjects exposed to I-NO, as shown in the table below.

Table 8.2.7.2.4 (from table 8.1.5.4.2) Incidence of subjects with pneumothoraces identified by investigators as adverse events in INO-01/ -02 and /-03 trials.

Adverse Event	Control n=41	I-NO 5 ppm n= 45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	I-NO Combined n=128
Pneumothorax	1 (2%)	4 (9%)	3 (7%)	3 (8%)	10 (8%)

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
3) Air Leak Syndrome (ALS) (cont)

Table 8.2.7.2.5 Listing of subjects with pneumothoraces identified as adverse events by investigators in the INO-01/ -02 trial[a].

Subject group	Duration of tx	Outcome (28 days)
Control		
02-04001	204	Seizures, BPD[b]
I-NO 5 ppm		No ECMO, HFOV/HFJV
01-03024	2	ECMO
01-03028	100	None[c]
01-05002	21	Seizures, ECMO
01-11012	10	Died
I-NO 20 ppm		
01-03025	113	Died
01-11005	70	Died
02-17001	98	None[c]
I-NO 80 ppm		
01-06006	144	Died
01-11011	62	Died
02-15003	10	None[c]
02-04004	6	None[c]

a. Data from electronic datasets.
b. Broncho-pulmonary dysplasia (BPD).
c. No death, ECMO, seizures or BPD.

The sponsor submitted case report from for the following subjects, identified by the sponsor as having had a pneumothorax in the INO-01/ -02 trial. Note that one individual, 02-15003, is listed in the electronic dataset, but not submitted by the sponsor. This discrepancy was not resolved.

Table 8.2.7.2.6 Listing of pneumothoraces in the INO-01/ -02 trial[a].

Subject group	Duration of I-NO therapy	Outcome (28 days)
Control		
01-03004	119	ECMO
01-03006	17	ECMO, seizures, BPD
01-05001	2	ECMO
01-07001	2	BPD
02-04001	204	Seizures, BPD
02-12002	3	ECMO
I-NO 5 ppm		
01-03002	19	None[c]
01-03024	2	ECMO
01-03028	100	None
01-05002	21	Seizures, ECMO
01-06002	51	Seizures, BPD, RAD[b]
01-11012	10	Died
02-13003	8	BPD, ECMO
I-NO 20 ppm		
01-03024	113	Died
01-11001	5	ECMO
01-11005	70	Died
02-17001	98	None
I-NO 80 ppm		
01-06003	8	Data Missing
01-06006	144	Died
01-11006	52	Seizures, ECMO, BPD
01-11011	62	Died
02-04004	6	None

a. Case report forms submitted at reviewer's request.
b. Reactive Airways Disease (RAD).
c. No death, ECMO, RAD or seizures.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
3) Air Leak Syndrome (ALS) (cont)

Overall, 5/21 subjects with pneumothorax were female (24%). This compares with the 37% of the control subjects and 46% of the I-NO subjects overall who were female. There were 9 whites (43%) and 8 blacks (38%). Overall, there were blacks composed approximately 20% of the population.

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

Table 8.2.7.2.7 (from table 6.0.4.13.1). Listing of pneumothoraces in the INO-03 trial[a].

Subject group	Duration of I-NO therapy	Notes
I-NO 5 ppm		No ECMO, HFOV/HFJV
03-57003	120	No ECMO, HFOV/HFJV
03-59004	32	No ECMO, HFOV/HFJV
I-NO 20 ppm		
03-58001	16 hours	ECMO
03-59002	168 hours	No ECMO, HFOV/HFJV Required O2 at 28 days, BPD

a. Data from NDA volumes 2.30 and 2.31.

Pneumothorax and pneumopericardium were also identified as Serious Adverse Events for several of the infants in the I-NO group, and in no control subject in the INO-01/ -02 trail.

Table 8.2.7.2.8 (from table 8.1.2.2.1 Serious Adverse Events from the INO-01/ -02 study). Serious adverse events within the Air Leak Syndrome.

Study Group	Subject #	Adverse Event	Withdrawn from Study?	Outcome	Related to Drug[a]?
Control	None				
I-NO 5 ppm	01-11012	Pneumothorax	No	Died	Not Related
I-NO 20 ppm	01-03025	Pneumothorax	No	Sequelae	Not Related
	01-11005	Pneumothorax Pneumopericardium	No	Died	Not Related
I-NO 80 ppm	01-06006	Tension pneumothorax	No	Died	Not Related
	01-11011	Bilateral pneumothoraces Pneumopericardium	No No	Died Died	Remotely Related Remotely Related

a. Relationship to I-NO administration per principle investigator.

Five of the 10 infants who died in the I-NO group also had one of the components of the ALS. The two infants in the control group who died (one during the trial, one after discharge) did not have ALS. With the exception of the first infant listed below, the subjects all received I-NO for an extended period of time. Note that patient #5 was receiving I-NO at the time of the pneumothorax and pneumopericardium which precipitated his death.

Table 8.2.7.2.9 Deaths with air leak syndrome adverse events in the INO-01/ -02 trial[a,b].

Subject	Duration of study gas (hrs)	Air Leak Syndrome?[b]	Refractory Hypoxemia	Support Withdrawn?	Notes
Control					
I-NO 5 ppm					
01-11012	10	Yes	Yes	Yes	
I-NO 20 ppm					
01-11005	56	Yes	Yes	No	
01-11015	120	Yes	Yes	Yes	Died after 32 days First pneumothorax occurred Pre-I-NO
I-NO 80 ppm					
01-06006	144	Yes	Yes	No	Persistent methemoglobinemia
01-11011	60	Yes	No	No	Pneumopericardium

a. Data from section 8.1.1 and electronic datasets.
b. One other subject in the 20 ppm I-NO group, 01-11015, had a pneumothorax at birth, and died after 32 days.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
3) Air Leak Syndrome (ALS) (cont)

Patient deaths with Air Leak Syndrome from the INO-01/-02 trial

1) Patient 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 progressive hypoxemia. A decision was made to withdraw therapy, and the subject died 21 days after start of study gas.

2) Patient 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 26 days after ending I-NO, after developing a pneumothorax.

3) Patient 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.

4) Patient 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.

5) Patient 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.

Patient deaths with Air Leak Syndrome from the INOSG trial (data from individual case summaries)

1). Patient Buf-8 received control gas, and died after support was withdrawn due to 'heart failure.' He is also listed as having a pneumothorax within the first 7 days of life, but no other details are available.

2). Patient UT@D-1 received I-NO, and is reported to have died as a result of a pneumomediastinum after 3 days on I-NO. Her methemoglobin level was 13.2% after 12 hours, and 8.7 after 24 hours. That is the last recorded methemoglobin level prior to the development of pneumomediastinum and death. No NO_2 levels were collected.

No increased risk for pneumothoraces or 'Air leak syndrome' was identified in any study in the secondary database.

The possible mechanism for such an effect of I-NO is the same mechanism posited for the other acute and chronic pulmonary toxicities associated with I-NO above: the formation of reactive intermediates between I-NO and O_2, followed by nitrosylation of structural proteins, leading to decreased elasticity.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
3) Air Leak Syndrome (ALS) (cont)

Conclusions

First, notice that the subjects in the NINOS trial had a much lower incidence of Air Leak Syndrome than those in the INO-01/ -02 trial (both control and I-NO groups). Why this disparity exists is not clear, since the infants in the INO-01/ -02 trial had a significantly lower OI at entry, suggesting they were less severely ill. There was also a longer period of exposure to gas in the NINOS trial (see table 8.0.3.2 above), and a similar proportion of infants in both trials who received HFOV or HFJV (see NDA volume 2.14, table 36 and volume 2.17, table 22).

Second, the number of pneumothoraces identified as adverse events was increased in the I-NO group in the INO-01/ -02 trial (2% versus 8%). In the NINOS, there was also an increased % of subjects who developed air leak syndrome during administration of I-NO. No difference in the rate of ALS or pneumothorax exists in the larger follow-up databases, although there was an increase in ALS in the NINOS trial during I-NO administration. It is possible that not all pneumothoraces were identified as adverse events by investigators. It is unlikely that somehow there was disagreement about how to define an event as a pneumothorax, and Air leak syndrome was defined the same in both studies. As discussed above, data on the two adverse events (pneumothoraces and Air Leak Syndrome) were collected by two separate individuals: adverse events during the trial were identified by the principle investigator; Air Leak Syndrome (ALS) was collected at the end of the trial by the medical monitors of the trial. In this case, the two individuals collected varying data on the incidence of an adverse event. Unfortunately, the individuals identified by the medical monitors were not individually collected, so no review of the individual patient case report forms is possible at this time.

Pneumothorax was identified as a serious adverse event only in the subjects in the I-NO group. Overall, 4 of these 11 subjects who had pneumothorax identified as an adverse event in the INO-01/ -02 trial died (36%). This population, then, had a significantly higher mortality rate (36%) than the overall population of infants (13.2% for the controls, 10.3% for the I-NO group, see table 8.1.1.1). All four individuals who died were in the I-NO group. One of these subjects died while being administered I-NO (subject #01-11011).

Finally, several infants who died experienced pneumothoraces. Five individuals in the I-NO group in the INO-01/ -02 trial who died also had components of the ALS. The fifth individual, #01-11015, had a pneumothorax before starting I-NO, and so was not counted as an emergent adverse event by the investigators. Four of the five received I-NO for prolonged periods (ranging up to 144 hours). No control infant who died also had ALS. Similarly, in the INOSG trial, one infant who received I-NO died of a pneumomediastinum (UT@D-1). This child also had elevated methemoglobin levels (13.2 after 12 hours and 8.7% after 24 hours, the last value prior to death). One control infant in the INOSG trial who died also had a pneumothorax, but this was not the cause of death (Buf-8).

Finally, abnormal nitrosylation of proteins through the production of reactive intermediates can also contribute to damage of the pleural proteins, leading to decreased pulmonary elasticity and increased risk for pneumothorax. This was the same mechanism proposed for the possible increased risk of Reactive Airways Disease above.

In conclusion, the database suggests that it is possible that a relationship exists between I-NO administration and pneumothoraces. The association between I-NO and the other components of the Air Leak Syndrome (pneumomediastinum and interstitial edema, pneumopericardium, and interstitial emphysema) cannot be determined from this database. No dose-effect can be determined. There is also not sufficient data to determine whether the increased % of blacks who had pneumothoraces is clinically significant. For the purposes of this safety review, there is a possible association between I-NO administration and the development of pneumothoraces.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO (cont)

4) Chronic Pulmonary Injury

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. The shading emphasizes the two clinical events which differed in their rates between control and I-NO subject. First, increased numbers and % of infants who received I-NO required supplemental O_2 during the first year of life, compared with the control infants. Second, the infants who received I-NO reported fewer URIs during the first year of life.

Table 8.2.7.2.10 (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 (10%)	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.

Of the infants whose caregivers answered yes to using supplemental O_2 during the first year of life (all in the I-NO group), none required O_2 at the end of one year. The remainder used O_2 for periods up to 11 months after 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. All but one of these individuals was on O_2 at the time of their initial discharge from the hospital. One subject who did not require O_2 at the time of discharge was on O_2 at the one-year follow-up (01-07002).

Not all of these infants have available follow-up, however, as shown in the table below.

Table 8.2.7.2.11 (from table 6.0.3.13.3.9) Details of follow-up for infants requiring supplemental O_2 at time of discharge and up to 1 year of age for infants with known follow-up in INO-01/-02[a].

Infants requiring supplemental O_2	Control	Missing Data[b]
At time of discharge		
Control	6/41 (15%)	0
I-NO 5 ppm	9/41 (22%)	0
I-NO 20 ppm	3/33 (9%)	0
I-NO 80 ppm	6/36 (17%)	0
Combined I-NO	18/110 (16%)	0
During 1 year follow-up of same subjects		
Control	0/2%)	4
I-NO 5 ppm	8/9 (89%)	1
I-NO 20 ppm	1/3 (33%)	0
I-NO 80 ppm	3/6 (50%)	1
Combined I-NO	12/18 (67%)	2

a. From individual case report forms for long-term follow-up, Volumes 7.1 through 7.12, and from electronic datasets.
b. Missing data in the form of case report form for the 1 year follow-up examination, including details of the respiratory system review.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
4) Chronic Pulmonary Injury (cont)

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, and the number of months they required O_2 after discharge. Those infants with available records are shown as 'Unknown. 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.

Table 8.2.7.2.12 Subject in the INO-01/ -02 trial who required supplemental O_2 at 1 year follow-up[a].

Subject #	Duration of study gas	Peak NO_2 level	Peak MetHgb level	Received HFOV/HFJV?	Received Surfactant?	Months on Supplemental O2
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.

Subjects without a 1 year follow-up, who required O_2 at the time of discharge, are recorded below.

APPEARS THIS WAY
ON ORIGINAL

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
4) Chronic Pulmonary Injury (cont)

Table 8.2.7.2.13 Subject in the INO-01/ -02 trial who required supplemental O_2 at time of discharge, without available data at 1 year follow-up[a].

Subject #	Duration of study gas	Peak methemoglobin level	Peak NO2 level
Control group			
01-03006	7	0.1	0.6
01-03019	16	0.6	0.2
02-04001	200	0.5	0.1
02-12002	3	0.7	0.0
I-NO 5 ppm			
02-13003	8	6.2	1.9
I-NO 20 ppm			
I-NO 80 ppm			
01-03029	123	3	11.9
02-07005	14	2.9	3

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

The INOSG study report also measured the number of days of supplemental O_2 used by each subject. One infant in the control group required supplemental O_2 for 445 days, including 405 days of mechanical ventilation. No case report form or other information regarding this individual (subject UCSF-10) is available. Excluding that subject, the average need for supplemental O_2 was 19±21 days in the control group and 11±6 days in the I-NO group (p value =0.066).

The potential mechanism for a chronic damage to the pulmonary system by I-NO is not established, damage to pulmonary proteins, discussed for the acute bronchospastic effects of I-NO discussed above, could also lead to significant long-term pulmonary effects. In this regard it is reasonable to compare nitrosylation of proteins to the effects of abnormal glycation of proteins in the vessels of diabetics. The consequences are abnormal protein-protein binding via the reactive intermediates, leading to deposited abnormal proteins which are resistant to degradation. These proteins disrupt the normal architecture of the vessel wall, and may play an important role in the pathology of diabetic vascular disease. In the lung, abnormal nitrosylation of long-lived proteins might similarly alter their architecture, with significant clinical consequences. Such a mechanism, of course, remains speculative at this point.

Conclusion

The only adverse event at the one-year follow-up visit with a significantly different incidence between control and I-NO groups in the INO-01/ -02 trial is the % of infants who received home O_2 during the one year following discharge. No control subject in the follow-up is recorded as receiving, while 14% of the combined I-NO subjects used O_2 (and 70% of the I-NO subjects who were discharged on O_2). Note that 4/18 infants in the I-NO group, and 2/2 infants in the control group who were receiving O_2 at the time of discharge with available records did not use it during the next 1 year. One way to interpret this is that these infants improved, while some chronic injury (perhaps associated with I-NO administration) slowed improvement in the other infants. Also note that one individual who received I-NO 5 ppm was discharged without requiring O_2, but required it during the 1 year follow-up. This suggests that a progressive injury due to I-NO, one not apparent at time of discharge, may occur.

The data do not allow us to look at the effect of increased methemoglobin or NO_2 concentrations. 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 01-07005, are likewise missing follow-up data.

There is no suggestion that a higher percentage of the infants in the I-NO group received either HFOV/HFJV or surfactant. This is relevant as the higher pulmonary pressures used in HFOV/HFJV could be a separate mechanism for pulmonary injury. The use of surfactant might be expected to ameliorate pulmonary injury. One caveat to this interpretation is that only 2 of the 6 infants in the original control group have follow-up data available. A single infant in the control group requiring O_2 after 1 year might lessen, but not eliminate, the level of concern.

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
4) Chronic Pulmonary Injury (cont)

The duration of exposure to I-NO, and the maximum methemoglobin and NO_2 levels do not show any clear association with the infants who needed O_2 during the first year after discharge. Of the three individuals with the longest duration of exposure to I-NO, one died (01-11015), one is missing follow-up data (01-03029) and one required O_2 for 4 months (02-14001). This could suggest either that the injury is independent of dose and duration of exposure, or that only a fraction of the infants requiring home O_2 had been injured by I-NO.

In the absence of other data, however, these data argue for a possible link between I-NO administration and chronic pulmonary injury, as marked by continued O_2 dependence. It is also possible that the injury may not manifest itself until after discharge (per patient 01-07002). For the purposes of this safety review, there is a possible link between I-NO administration and long-term requirement for supplemental O_2.

5) Decreased PaO_2 as a result of decreased FiO_2 during I-NO administration

Because the introduction of study gas meant that the delivered O_2 decreased from 100% to 90%, the INOSG trial measured two sets of blood gases. After the first set was taken, the FiO_2 was reduced from 100% to 90%, and a second set obtained 15 minutes later. If the subject did not tolerate the decrease (FiO_2 falls >15%), they were counted as a treatment failure, and were returned to 100% FIO_2. The results are shown in the table below. No information is available regarding any subjects who were withdrawn for lack of tolerance to 90% O_2.

Small (albeit statistically significant) changes occurred in PaO_2 between Baseline 1 and Baseline 2 values. No effect on pH or pCO_2 was detected.

Table 6.0.2.12.2.14 Effect of lowered FIO_2 on pulmonary parameters in INOSG.

Parameter	Control Group			I-NO Group			
	Baseline 1	Baseline 2	Change	Baseline 1	Baseline 2	Change	p value[a]
PaO_2	38±9.1	39.5±9	1.5	41.3±9.4	39.8±9.8	-1.5	0.001
pH	7.47±0.14	7.49±0.13	0.03	7.50±0.12	7.52±0.11	0.02	0.725
$PaCO_2$	33±11	28±32	-1.7	32.4±12	31.5±11	-0.93	0.71
Pre-Ductal O_2/ $TCPPO_2$ (%)	88.6±9.6	88.5±9.8	-0.08	85.9±11.6	86.2±10	0.3	0.67
Post-Ductal O_2/ $TCPPO_2$ (%)	84.4±10	85.1±10	1.0	82.4±16	83.1±14	0.76	0.825

a. Wilcoxon Rank Sum test used to compare median differences between control and I-NO groups.
b. the Baseline 1 value for the I-NO groups is higher than for the control group (p=0.061 using non-paired t-test).

8.2.7.2 Respiratory System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO
5) Decreased PaO_2 as a result of decreased FiO_2 during I-NO administration (cont)

In the long-term, this effect of the decreased FiO_2 was overcome by the effect of I-NO to increase oxygenation, as discussed in section 7.0.3.

Conclusion

For the purposes of this review, the decreased FiO_2 delivered to the subjects as a part of the delivery of I-NO, was associated with a small, significant decrease in PaO_2. This effect is more than compensated for by the effect of I-NO to improve oxygenation relative to control gas.

8.2.7.3 Respiratory System Adverse Events Considered Unlikely to be Related to I-NO

1) CO_2 Retention

No adverse event related to CO_2 retention by an investigator in the INO-01/ -02 and /-03 trials.

The table below summarizes the average effects of I-NO on pCO2 in the NINOS, INOSG and INO-01/ -02 and /-03 trials. There was no evidence of CO_2 retention in the mean values. No subject had therapy altered by increased pCO_2.

Table 8.2.7.3.1 Acute effects of I-NO on mean pCO_2 in the INOSG and INO-01/ -02 trials.

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
Total subjects	69	41	36	67	141
INOSG[b]					
Baseline	33.5±11			32.4±12	
After 20 minutes of study gas	32.7±12			28.4±10	
Change from baseline after 20 minutes of study gas	-0.9±5			-4.1±8	
INO-01/ -02[c]					
Baseline	32.8±10.4	29.3±8.2	32.0±11	29.5±8	30.2±9
After 30 minutes of study gas	32.1±11	28.0±8	30.7±11	28.2±9	28.9±10
Change from baseline after 20 minutes of study gas	-0.75±5	-1.24±6	-1.31±4	-1.17±5	-1.24±5

b. INOSG data from NDA, volume 2.16, Tables T-7. Baseline value is taken as the first baseline measurement.
c. INO-01/ -02 data from NDA volume 2.18, Table T-13.

Next, the INOSG and INO-01/ -02 individual data was examined, and any subject whose pCO_2 rose> 5 mmHg from baseline to 0.5 hours was tabulated. While the numbers are small, the individuals who had their pCO_2 rise acutely were also extremely sick, as marked by the degree of long-term residual effects they suffered. For instance, 6/9 subjects received ECMO.

Table 8.2.7.3.2 Subjects with acute increases in pCO_2 >5 mmHg from baseline to first follow-up value in the INOSG[b] and INO-01/ -02 trials[a].

Subject #	Baseline pCO_2	Post-study gas pCO_2	Notes
Control 2/69 (3%)			
01-03019	41	53	Sustained elevation in pCO2 Discharged with seizures and chronic lung disease (CLD) after ECMO
UTD@D-2	46	54	Received ECMO
I-NO 5 ppm 3/41 (7%)			
01-03024	36	41	Tx failure shortly after 30 minutes. Discharged after ECMO
01-04007	36	51	Sustained elevation in pCO2 Discharged with CLD after ECMO
01-05006	26	41	Sustained elevation in pCO2 Discharged after ECMO
I-NO 20 ppm 1/36 (3%)			
02-14007	48	56	Tx failure shortly after 30 minutes. Discharged with CLD
I-NO 80 ppm 3/67 (4%)			
02-11007	28	36	Sustained elevation in pCO_2 Discharged home
02-15003	43	49	Sustained elevation in pCO_2 Discharged home
Yale-1	31	52	Received ECMO
Combined I-NO 6/144 (5%)			

a. INO-01/ -02 Data from individual subject data tables, NDA volume 2.23, appendix 16.2.2.4. INOSG measured pCO2 after 20 minutes of study gas. INO-01/ -02 measured pCO2 after 30 minutes.
b. INOSG data from NDA volume 2.16, appendix 16.2.4.

8.2.7.3 Respiratory System Adverse Events Considered Unlikely to be Related to I-NO

1) CO_2 Retention (cont)

Conclusion

While there are individuals whose pCO_2 did rise acutely after administration of study gas, no rate difference between the control and I-NO subjects was detected. For purposes of this review, a link between I-NO and acute CO_2 retention is considered unlikely.

2) Need for supplemental O_2 at time of discharge

Since all of the children required O_2 during the early parts of their hospitalizations, need for supplemental O_2 was not identified as an adverse event by the investigators. However, data on the need for O_2 at time of discharge was collected prospectively.

There was no difference in the % of subjects who required supplemental O_2 at time of discharge in either the INO-01/ -02 or INOSG trials.

Table 8.2.7.3.3 Incidence of use of supplemental O_2 at time of discharge in the NINOS, INO-01/ -02 and /-03 and INOSG trials[a].

	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.

The list of subjects requiring supplemental O_2 at time of discharge is below, along with their treatment group, duration of exposure to study gas, and their maximum NO_2 and methemoglobin levels.

Table 8.2.7.3.4 Subject in the INO-01/ -02 trial who required supplemental O_2 at time of discharge[a].

Subject #	Duration of study gas	Peak methemoglobin level	Peak NO_2 level
Control group			
01-03006	7	0.6	0.1
01-03019	16	0.6	0.2
01-07001	2	0.4	0.4
01-09001	152	0.4	0.8
02-04001	200	0.5	0.1
02-12002	3	0.7	0.0
I-NO 5 ppm			
01-03017	18	0.5	0
01-04007	13	0.3	0.1
01-06002	51	5.6	2
01-07008	82	0.7	2
01-17005	155	1.1	0.2
02-12003	41	0.5	0.7
02-13003	8	6.2	1.9
02-14001	140	1.4	0
02-14002	98	1.6	0.1
I-NO 20 ppm			
01-04006	21	0.9	0.3
01-11015	144	0.7	0.1
02-14007	3	0.4	0
I-NO 80 ppm			
01-02003	9	3	2
01-03029	123	11.9	3
01-11006	52	3.8	2
02-12001	25	3.6	0.8
02-07005	14	2.9	3
02-14003	33	7.3	2
02-14005	18	3.6	1.6

a. Data from electronic datasets.

8.2.7.3 Respiratory System Adverse Events Considered Unlikely to be Related to I-NO (cont)

2) Need for supplemental O_2 at time of discharge (cont)

There was also no overall difference in the incidence of 'Chronic lung disease' in the NINOS trial, which includes the need for O_2 as well as an abnormal chest x-ray. In the group of individuals who received I-NO, 80 ppm, there was a non-significant increase in the % of infants with CLD. This analysis is complicated, because only those infants who did not respond fully to I-NO 20 ppm were enrolled in the 80 ppm group. They might represent a population with more respiratory damage at baseline. No individual patient data are available.

Table 8.2.7.3.5 Incidence of use of 'Chronic lung disease' at time of discharge in the NINOS trial[a].

Adverse Event	Control	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
Chronic lung disease[b]	15/121 (12%)	7/59 (12%)	9/54 (17%)	16/114 (14%)

a. Data from Table 6.0.1.13.1.2 above and electronic datasets.
b. Chronic lung disease defined as O2 >21% required at 28 days of age with abnormal chest x-ray.

Conclusion

Aside from the subjects in the NINOS trial who received I-NO 80 ppm, there was no difference in the rate of need for supplemental O_2 use between the control and I-NO subjects at the time of discharge. The subjects in the 80 ppm group of the NINOS were a selected subset of children who failed to respond to 20 ppm, and thus an especially 'sick' population. For purposes of this review, a link between I-NO and the need for supplemental O_2 at time of discharge is considered unlikely. Note that there was an association between I-NO administration and the use of O_2 after discharge, discussed above.

8.2.8 Dermatological

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

1) Rash.
2) Cellulitis.
3) Injection site reaction.

8.2.8.1 Adequacy of Development Program in Assessing Dermatological Risk for I-NO

Dermatological adverse events were collected as part of the overall adverse events in the INO-01/ -02 and /-03 trials, as seen in the table below. The number of subjects was 41 in the control and 128 in the I-NO group.

Table 8.2.8.1.1 (from table 8.1.5.4.2) Reported dermatologic adverse events from INO-01/ -02 and INO-03[a].

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
Injection site reaction		1 (2%)			1 (<1%)
Cellulitis			1 (2%)	1 (3%)	2 (2%)
Ecchymoses				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.

No labs, and no specific adverse events prospectively followed were identified in any trial.

1) Rash

The effect of I-NO on the incidence of rashes could not be assessed from the available data. There were no rashes identified as adverse events in the INO-01/ -02 and /-03 database, and no rashes associated with I-NO use have been reported in the secondary database.

8.2.8.2 Dermatological System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No adverse events were identified that fell into this category.

8.2.8.3 Dermatological System Adverse Events Considered Unlikely to be Related to I-NO

1)Injection site reaction

An injection site reaction occurred in 1 subject in the I-NO, 5 ppm, group.

Patient 01-6009 had a cutaneous reaction following two injection of intravenous calcium, and recovered with topical specific therapy.

Conclusion

This adverse event was not related to the adminstration of I-NO.

2) Cellulitis

Cellulitis was identified in 2 (3%) of the subjects in the INO-01/ -02 and /-03 database.

Patient 02-15006, who received I-NO 80 ppm, had a superficial phlebitis at an injection site, and recovered with specific therapy in 4 days.

Subject 02-04007, who received I-NO 5 ppm, had a candida albicans skin infection which required therapy for 14 days before a full recovery.

Conclusion

This adverse event was unlikely to be related to the adminstration of I-NO.

8.2.9 Special Senses

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

1) Changes in sense of taste
2) Changes in visual acuity
3) Changes in sense of smell
4) Changes in proprioceptive sense

8.2.9.1 Adequacy of Development Program in Assessing Special Senses 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. This includes special senses adverse events, as shown in the table below. For overall special senses adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO. The database is too small to comment on the relative incidence of overall special senses adverse events in the I-NO and control groups.

Table 8.2.9.1.1. (from table 8.1.5.4.2) Reported special senses 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
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.

Specific adverse events in the special senses system included audiologic examinations at the time of discharge and after 1 year for all surviving infants. At time of discharge, audiologic examinations were performed on A total of 36 controls and 98 I-NO subjects had available audiologic data at time of discharge. A total of 36 control and 95 I-NO subjects were available after 1 year for audiologic examination.

No labs were collected which were relevant to the special senses system.

The following adverse events cannot be assessed from this database, as no information regarding their occurrence was collected in the database:

1) Changes in sense of taste
2) Changes in visual acuity
3) Changes in sense of smell
4) Changes in proprioceptive sense

8.2.9.2 Special senses system Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No adverse events were identified which fell into this category.

8.2.9.3 Special Senses Adverse Events Considered Unlikely to be Related to I-NO

1) Changes in hearing acuity, both short- and long-term

In the adverse events for INO-01/ -02, one subject was identified by investigators as having a possible decrease in hearing acuity on the right side.

1) Subject 02-15003 received I-NO 80 ppm, and had a questionable loss of hearing on the right-side which resolved. The event occurred 18 days after subject was weaned from I-NO.

In the INO-01/ -02 trial, the subjects were tested for diminished audiologic acuity before discharge from the hospital. Note that not all subjects had hearing tested before discharge.

Table 8.2.9.3.1 (from table 6.0.3.13.1.1) Rates of sensorineural hearing loss at time of discharge from INO-01/ -02 trial[a].

Changes in safety endpoints	Control	Combined I-NO
Incidence of sensorineural hearing loss[b]	5/36 (14%)	16/98 (16%)

a. Sensorineural hearing loss was detected using brain stem auditory evoked responses (BAER) and measured after 28 days. Data from NDA volume 2.17, page 094708.

8.2.9.3 Special Senses Adverse Events Considered Unlikely to be Related to I-NO

1) Changes in hearing acuity, both short- and long-term

As part of the 1 year follow-up examination, hearing acuity was also assessed in the INO-01/ -02 trial.

Table 8.2.9.3.2 (from table 6.0.3.13.3.5) 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	I-NO combined
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

There is no data from the secondary database associating I-NO with acute hearing loss.

Conclusion

There is no evidence that exposure to short-term I-NO is associated with acute or long-term changes in hearing, as assessed by BAER. For purposes of this review, it is unlikely that I-NO administration is related to decreased hearing acuity.

8.2.10 Genitourinary

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

1) Kidney Failure.
2) Proteinuria.
3) Hematuria.
4) Pyuria.
5) Crystalluria.

8.2.10.1 Adequacy of Development Program in Assessing Genitourinary 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. This includes genitourinary adverse events, as shown in the table below. For overall genitourinary adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO. No differences in the rates of overall genitourinary adverse events were detected, although the number of such events is small.

Table 8.2.10.1.1 (from 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
Genitourinary system	1 (2%)		3 (7%)		3 (3%)
Kidney failure	1 (2%)		2 (3%)		
Kidney abscess		2 (5%)			2 (2%)
Urogenital anomaly			1 (2%)		
Acute tubular necrosis		1 (2%)			1 (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.

No specific adverse events were identified to be followed from the genitourinary system.

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 requested from the sponsor. Whenever available, this has been included in this review. For overall genitourinary adverse laboratory events (BUN, creatinine), the database includes 41 control subjects and 128 subjects exposed to I-NO.

No data on urinalyses were collected or submitted. This includes data on hematuria, pyuria, proteinuria, creatinine clearance, crystalluria or urinary tract infections.

The following adverse events routinely cannot be assessed from this database:
hematuria, pyuria, proteinuria, creatinine clearance, crystalluria or urinary tract infections

The following adverse events cannot be assessed for their relationship to I-NO administration from the database because no data was submitted regarding their occurrence:

1) Proteinuria
2) Hematuria
3) Pyuria
4) Crystalluria

8.2.10.2 Genitourinary System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No adverse events were identified in this category.

8.2.10.3 Genitourinary System Adverse Events Considered Unlikely to be Related to I-NO

1) Kidney failure

In the INO-01/ -02 and /-03 trials, four subjects were identified by investigators as having adverse events associated with kidney failure. One individual was categorized as 'Acute tubular necrosis,' while three were categorized with 'Kidney failure.'

Table 8.2.10.3.1 (from table 8.1.5.4.2) Reported adverse events related to kidney failure identified by investigators from INO-01/ -02 and INO-03 trials[a].

	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
Genitourinary system					
Kidney failure	1 (2%)		2 (3%)		2 (2%)
Acute tubular necrosis		1 (2%)			1 (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.

The following subjects were identified as having kidney failure or acute tubular necrosis as an adverse event in the INO-01/ -02 and /-03 trials.

1) Subject 02-15005, received control gas, and developed renal failure at approximately the same time. The infant was a treatment failure and ultimately died.

2) Subject 01-01008, received I-NO 5 ppm and developed renal failure which lasted 3 days at approximately the same time. The subject recovered and was discharged without ECMO or dialysis.

3) Subject 01-03025, received I-NO 20 ppm. The infant developed renal failure lasting 1 hour (!) before receiving I-NO, but ultimately worsened and died.

The fourth individual was not identified by the sponsor or in the electronic database.

The mean creatinine and BUN values from the INO-01/ -02 trial are shown below. There was a significant increase in the BUN in all groups from baseline to post-I-NO. There was no difference detected between the control and the I-NO groups.

Table 8.2.10.3.2 (from table 8.1.6.2.1.1.1) Mean BUN and creatinine values from INO-01/ -02[a].

Lab Test[c]	Placebo		5 ppm I-NO		20 ppm I-NO		80 ppm I-NO	
	Baseline	Post-study gas	Baseline	Post-I-NO	Baseline	Post-I-NO	Baseline	Post-I-NO
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)
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

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

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

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

8.2.10.3 Genitourinary System Adverse Events Considered Unlikely to be Related to I-NO

1) Kidney failure

The number of subjects who had abnormal BUN or creatinine was also tabulated from individual patient records. No data on the occurrence of dialysis for renal failure in any subject is available.

Table 8.2.10.3.3 Individuals with markedly abnormal post-study gas BUN or creatinine from INO-01/ -02 and /-03 trials[a,b].

Patient #	Lab Test	Baseline value	Post-I-NO value	Notes
Placebo				
01-07001	Creatinine	1.2	1.9	high
I-NO 5 ppm				
02-11004	BUN	8.7	18.2	high
I-NO 20 ppm				
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
I-NO 80 ppm				
01-03029	Creatinine	1.1	3.5	high

a. Data from NDA, volumes 2.25 and 2.31, individual patient listings
b. Serum creatinine was identified as markedly abnormal were >2X upper limits of normal value.

No follow-up information regarding these abnormal BUNs and creatinines is available.

As a marker for proximal tubular function, it is also worthwhile to examine the changes in serum phosphate, bicarbonate, and potassium. As noted above, no data was collected on either bicarbonate or potassium. The table below shows the average phosphate values in the INO-01/ -02 trial. In all groups except the I-NO 80 ppm group, there was a significant increase in phosphate in the follow-up lab.

Table 8.2.10.3.4 (from table 8.1.6.2.1.1.1) Mean serum phosphate values from INO-01/ -02[a, c].

Lab Test[c]	Placebo		5 ppm I-NO		20 ppm I-NO		80 ppm I-NO	
	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

a. Source: NDA volume 2.50, pages 341010-341510 and volume 2.25.
b. Per protocol, follow-up labs were to be taken no more than 12 hours after end of exposure to treatment gas.
c. Data shown as mean±standard deviation (# of subjects with data). Shaded boxes indicate that baseline and post-study gas labs differ significantly using 2-sided unpaired t test.

The next table shows those individuals in the INO-01/ -02 with markedly abnormal serum phosphates.

Table 8.2.10.3.5 (from table 8.1.6.2.2.1a.1) Individuals with markedly abnormal post-I-NO phosphates 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
I-NO 5 ppm	No subjects			
I-NO 20 ppm				
01-03020	Phosphate	2.7	2.5	low
I-NO 80 ppm	No subjects			

a. Data from NDA, volumes 2.25 and 2.31, individual patient listings
b. Serum phosphates identified as markedly abnormal were <0.5X lower limit of normal on post-I-NO value.

8.2.10.3 Genitourinary System Adverse Events Considered Unlikely to be Related to I-NO

1) Kidney failure (cont)

One subject in the INO-01/ -02 trial died with renal failure.

1) 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.

Conclusion

There is no data to suggest an association between I-NO administration and renal failure, as defined by an increased BUN or creatinine. This use of BUN and creatinine as the only markers for renal failure is problematic, as it does not address the potential for nephrotic syndrome or other diseases where glomerular filtration rates are normal, when other aspects of renal function are impaired. The only marker for tubular function available for review is serum phosphate, where control and I-NO groups were similar. There is certainly precedent for non-glomerular injury due to inhalants (i.e., fluranes).

With regard to acute glomerular injury, it is unlikely that there is an association between the administration of I-NO and renal failure, reflected by increases in serum BUN and creatinine. The data are inadequate to determine if non-glomerular renal injury due to I-NO occurs.

8.2.1.1 Miscellaneous Adverse Events

The following miscellaneous potential adverse events were identified from the secondary sources or are adverse events normally explored as part of a safety review:

1) Allergic reaction.

8.2.11.1 Adequacy of Development Program in Assessing Miscellaneous System 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. This includes miscellaneous adverse events, as shown in table 8.5.4.2 above (categorized under body as a whole). For overall miscellaneous adverse events, then, the database includes 41 control subjects and 128 subjects exposed to I-NO.

No specific adverse events were identified to be followed prospectively in the miscellaneous system.

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 requested from the sponsor. Whenever available, this has been included in this review. For overall miscellaneous adverse laboratory events (e.g., eosinophilia suggesting allergic reaction), then, the database includes 41 control subjects and 128 subjects exposed to I-NO.

The following adverse events cannot be assessed for their relationship to I-NO administration from the database:

1) Allergic reaction

Allergic reactions were not identified as an adverse event in any trial.

8.2.11.2 Miscellaneous System Adverse Events Considered Possibly, Probably, or Definitely Related to I-NO

No adverse events were identified in this category.

8.2.11.3 Miscellaneous System Adverse Events Considered Unlikely to be Related to I-NO

No adverse events were identified in this category.

8.3 Summary of Key Adverse Findings/ Safety Summary

In this section, the adverse events which were identified above as either possibly, probably or definitely linked to exposure to I-NO will be listed, along with a reference to the relevant parts of section 8.2.

Table 8.3.1 Adverse events identified in section 8.2 as possibly, probably, or definitely linked to the administration of I-NO[a].

Body System	Adverse Event	Further Information
Cardiovascular system	Hypotension/Hypertension Bradycardia	Section 8.2.1.2
Gastrointestinal system	Hepatotoxicity, evidence by elevated LFTs	Section 8.2.2.2
Hemic & Lymphatic system	Elevated methemoglobin levels Elevated NO_2 levels Eosinophilia Neutropenia	Section 8.2.3.2
Metabolic & Endocrine system	None identified	
Musculoskeletal system	None identified	
Nervous system	None identified	
Respiratory system	Increased hypoxia & pulmonary hypertension after I-NO withdrawal Air leak syndrome[d] Acute reactive airways disease[b] Chronic pulmonary injury/ O_2 dependence[c]	Section 8.2.7.2
Special senses system	None identified	Section 8.2.8.2
Genitourinary system	None identified	Section 8.2.9.2
Miscellaneous system	None identified	Section 8.2.10.2

a. See section 8.2 for details of individual adverse events.
b. Acute in this term indicates reactive airways disease detected up to the time of the subject's initial discharge from the hospital.
c. Chronic in this term indicates damage detected at the 1-year follow-up visit.
d. Air-leak syndrome refers to pneumothoraces, pneumomediastinum, interstitial emphysema, pneumopericardium.

8.3 Summary of Key Adverse Findings (cont)

Table 8.3.2 Adverse events identified in section 8.2 for which inadequate data exists to determine their association to the administration of I-NO[a].

Body System	Adverse Event	Further Information
Cardiovascular system	Changes in cardiac output	Section 8.2.1.1
	Arrhythmias	
	Acute myocardial injury	
Gastrointestinal system	None identified	Section 8.2.2.1
Hemic & Lymphatic system	None identified	Section 8.2.3.1
Metabolic & Endocrine system	Hyper- / Hypo-kalemia	Section 8.2.4.1
	Hyper- / Hypo-natremia	
	Hyper- / Hypo-calcemia	
	Acid-base disturbances	
Musculoskeletal system	Acute or chronic muscle injury /rhabdomyolysis	Section 8.2.5.1
Nervous system	Sedation	Section 8.2.6.1
Respiratory system	Surfactant dysfunction	Section 8.2.7.1
Dermatological system	Rash	Section 8.2.8.1
Special senses system	Changes in sense of smell	Section 8.2.9.1
	Changes in visual acuity	
	Changes in sense of taste	
	Changes in proprioceptive sense	
	Changes in sense of touch	
Genitourinary system	Proteinuria	Section 8.2.10.1
	Hematuria	
	Pyuria	
	Crystalluria	
Miscellaneous system	Allergic reaction	Section 8.2.11.1

a. See section 8.2 for details of individual adverse events.

8.3 Summary of Key Adverse Findings (cont)

Table 8.3.3 Adverse events identified in section 8.2 that are unlikely to be associated with the administration of I-NO[a].

Body System	Adverse Event	Further Information
Cardiovascular system	Aortic valve vegetation Aortic thrombosis	Section 8.2.1.3
Gastrointestinal system	Gastrointestinal bleeding	Section 8.2.2.3
Hemic & Lymphatic system	Thrombocytopenia Altered coagulation parameters/ increased clinically significant bleeding	Section 8.2.3.3
Metabolic & Endocrine system	Hyper- hypo-glycemia	Section 8.2.4.2
Musculoskeletal system	None identified	Section 8.2.5.3
Nervous system	Intracranial hemorrhage Seizures Brain infarct Long-term mental development[b] Long-term psychomotor development[b] Long-term neurologic development[b] Long-term incidence of cerebral palsy[b]	Section 8.2.6.3
Respiratory system	CO_2 retention Acute need for supplemental O_2[d]	Section 8.2.7.3
Dermatological system	Injection site reaction Cellulitis	Section 8.2.8.3
Special senses system	Changes in sense of hearing, acute and chronic[e]	Section 8.2.9.3
Genitourinary system	Kidney failure (acute glomerular injury)	Section 8.2.10.3
Miscellaneous system	None identified	Section 8.2.11.3

a. See section 8.2 for details of individual adverse events.
b. In this term, long-term refers to abnormalities tested at the one-year follow-up.
d. Acute refers to need at time of initial discharge from the hospital.
e. Acute refers to need at time of initial discharge from the hospital, while chronic refers to abnormalities tested at the one-year follow-up.

Of the adverse events which were possibly, probably, or definitely related to the administration of I-NO, five are of the most concern to this reviewer: elevated methemoglobin levels; elevated NO_2 levels; increased air leak syndrome; increased reactive airways disease at time of discharge; and increased need for supplemental O_2 after discharge.

As discussed above, there is a definite link between I-NO administration and increased methemoglobin and NO_2 levels. In particular, the use of 80 ppm I-NO is tightly linked to both the development of increased methemoglobin and NO_2 levels, and the need for withdrawal of subjects from I-NO administration. No clinically relevant adverse event could be with the elevated methemoglobin or NO_2. This may be due to the small number of subjects, or to the protocol, which called for the discontinuation of subjects with persistent elevations of either methemoglobin or NO_2.

The second major area of safety concern is the possible pulmonary toxicity of I-NO. Several pieces of evidence, from different sources, suggest that exposure to I-NO may have adverse pulmonary consequences. First, the data from INO-01/ -02 suggest that there may be an increase in reactive airways disease in the period during and after exposure to I-NO. This agrees with the data in the literature about the acute effects of I-NO in adults. Second, the increased rate of pneumothoraces in infants exposed to I-NO in the INO-01/ -02 trial raises the possibility of more serious damage to the respiratory tract. Finally, the need for O_2 after discharge by the infants exposed to I-NO argues for a potential chronic pulmonary injury that does not resolve by the time the infants are discharged.

8.3 Summary of Key Adverse Findings (cont)

The INO-01/ -02 database also suggests that the consequences of pneumothoraces for infants who receive I-NO may be more severe that for control infants. First, one control and 11 I-NO subjects had a pneumothorax as an adverse event. Of these infants, 4 died, all of them in the I-NO group.

There is also is an attractive, but unproven, mechanism for this potential toxicity of I-NO. This was discussed in section 8.2.7.2 above. In short, the interaction of NO_2 and O_2 in the lungs of the infants would yield reactive intermediates which could cause nitrosylation of pulmonary and pleural proteins, altering pulmonary function acutely (leading to increased airway reactivity and decreased pleural elasticity/increased pneumothoraces) and chronically (pulmonary injury and scar formation, leading to impaired oxygenation and need for supplemental O_2) (4, 19, 54). Monitoring the NO_2 levels in the exhaled air may be inadequate to detect increased NO_2 formation, since the majority of NO_2 is trapped in the lung, this effect may not be reflected in increased NO_2 levels measured in the expired air. Overall, there is a possible and plausible link between I-NO administration and short- and long-term pulmonary damage. The consequences of this damage may be serious, including a possible link to serious adverse events and subject death.

Many of the remaining adverse events which are possibly linked to the administration of I-NO are laboratory abnormalities. For these events, inadequate follow-up exists to characterize their resolution in the particular subject. Based on the available data, there were no individuals with these abnormalities who had an adverse clinical outcome whose course was altered by a given lab abnormality.

The potential cardiovascular effects of I-NO, including the rebound hypoxia following I-NO weaning, all have the potential for serious adverse effects (for example, see subject 12-A01 in the NINOS trial). These reactions were infrequent in the database, and do not allow an examination of dose-dependence. The largest decreases in PaO_2 following I-NO withdrawal occurred during discontinuation of I-NO (see table 8.1.9.2.1).

Finally, there are several relevant adverse events for which inadequate data exists in the database. These are listed in table 8.3.2.

9.0 Labeling Review

While the proposed label will not be examined in detail (since the NDA application was withdrawn) some points regarding the proposed dose of I-NO needs to be discussed.

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

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

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

No drug interactions were identified.

1. I-NO dosage

The dose range proposed for I-NO is from 20 to 80 ppm. This is based first on the observation that there is a dose-dependent relaxation of the pulmonary vascular resistance from I-NO 5 ppm to doses >100 ppm. Second, in the INO-01/ -02 trial, there was a dose-dependent decrease in the % of subjects who received ECMO up to 80 ppm (see Table 6.0.3.12.2c.3).

Counterbalancing this data are the results of the NINOS trial. There, subjects with less than a full response to I-NO 20 ppm were no more likely to have a response to 80 ppm than the placebo patients were to respond to 'high-flow oxygen' after a less than full response to 'low-flow oxygen.' (see Table 6.0.1.12.3c.1 in this review and NDA volume 2.14, page 029808). This is similar to what has been reported in the literature: if low-dose I-NO dose not improve oxygenation, high-doses are unlikely to be effective(46).

Other authors have also reported that there was no clinical difference between the effect of low (5-20 ppm) and high (>20 ppm) I-NO in hypoxic neonates(47) (34) (41).

In the absence of superior efficacy, the safety profile of I-NO 80 ppm is also of concern. As discussed above, there is a definite link between I-NO 80 ppm and elevations in both methemoglobin and NO_2. In the context of the possible long-term toxicity of I-NO, and it's link to NO_2 levels, any exposure to I-NO 80 ppm exposes the infants to increased NO_2 concentrations, and potential short- and long-term pulmonary toxicity.

Proposal

The dosage of I-NO in all cases should be limited to 20 ppm. Monitoring should continue to be performed for methemoglobin and NO_2 levels.

9.0 Labeling Review (cont)

2. Drug interactions

No drug interactions were identified by the sponsor, and no interactions were identified in this safety review. Overall, however, the database is too small to allow for any assessment of the potential interactions. For instance, the potential interaction of I-NO with conventional bronchodilators cannot be determined.

Proposal

A statement on any label should alert the clinicians as to the lack of firm data about potential drug interactions.

10.0 Conclusions

As discussed in the summary of the efficacy data, section 7.5, the data supports the contention that I-NO administration is associated with a significant decrease in the use of ECMO. This effect of I-NO to decrease ECMO use may well be a reflection of its acute to improve oxygenation, rather than due to any other beneficial effect on the disease course. In support of this contention, no beneficial effect of I-NO on mortality or any other clinical endpoint was demonstrated, or even suggested, by the data. In the larger context, mortality rates for PPHN have been falling significantly from approximately 50-60% in the 70s and 80s, to approximately 15-20% today. This has been accomplished without the use of I-NO, suggesting that other therapies (HFOV/HFJV, surfactant, alkalinization, improved ICU monitoring) are having a positive impact on neonatal hypoxic respiratory failure. The falling mortality rate also mitigates against the argument that no effective therapy for PPHN now exists, so that a less than overwhelmingly effective drug might still require approval. It also means that the safety of I-NO needs to be firmly established prior to wide-spread use.

Unfortunately, the safety database has problems which severely limited the ability of the reviewer to detect adverse events associated with I-NO administration. The first, major difficulty with the safety database was the inadequacy of the data collected, and the small number of subjects followed. For most adverse events, the INO-01/ -02 and INO-03 trials were the only source of information. Given the baseline differences between the subjects in the INO-01/ -02 and NINOS trials, extrapolating between the two populations is difficult, and open to serious errors of omission due to inadequate data.

Additionally, the available safety data raise several potential safety issues. The most troubling of the adverse events associated with I-NO are the possible acute and chronic pulmonary toxicities: acute bronchospastic lung disease; Air Leak Syndrome; and long-term need for supplemental O_2. This association, like all of the safety data, relies on small numbers of subjects, and it is absolutely fair to say that there is no proof of a causative role for I-NO in these adverse events. In this reviewer's opinion, the association is possible and plausible, given the available data.

The definite association of I-NO, particularly the 80 ppm dose, with both methemoglobinemia and elevated NO_2 levels, is also a significant safety concern. Whether NO_2 has an etiologic role in the possible pulmonary toxicities of I-NO cannot be determined with the available data. The potential toxicity of NO_2 has been well-documented in the adult population, and there is no reason to think this toxicity would be less in a neonatal population with injured lungs. With regard to the demographics of adverse events, 8% of the white males had methemoglobin identified as an adverse event in the INO-01/ -02 and /-03 trials, while they made up approximately 50% of the subjects in the I-NO 80 ppm group. No other striking difference was noted between the occurrence of an adverse event and the demographics of the study.

Several other adverse events were also possibly linked to the administration of I-NO. The data are insufficient to determine the seriousness of these potential adverse events, or even to determine their duration or dose-response for most events. There is a strong suggestion that I-NO use is associated with eosinophilia which is I-NO dose-dependent.

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

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

In conclusion, then, there is not sufficient data suggesting a clear beneficial effect of I-NO on hard-endpoints (death, days of hospitalization, days of ventilation, incidence of chronic lung disease or neurological sequelae), in an era with other effective therapies and falling mortality rate. This is to be coupled with the potential adverse events associated with I-NO administration, and the inadequacy of the safety database for certain key adverse events. In the balance, the balance, the database is inadequate to support the safe and effective use of I-NO.

11.0 Recommendations and Reviewer Signature

NDA 20-845, inhaled nitric oxide for neonates with hypoxic respiratory failure, should not be approved.

/S/

Douglas C. Throckmorton, M.D.

12. References

1. Group NINOS. Inhaled nitric oxide in full-term infants and nearly full-term infants with hypoxic respiratory failure. *NEJM.* 1997;336:597-604.
2. Roberts JDJ, Fineman JR, Morin FC, Zapol WM. Inhaled nitric oxide and persistant pulmonary hypertension of the newborn. *NEJM.* 1997;336:605-610.
3. Kinsella JP, McCurnin DC, Clark RH, Lally KP, Null DM, Jr. Cardiac performance in ECMO candidates: echocardiographic predictors for ECMO. *J Pediatr Surg.* 1992;27(1):44-7.
4. Frostell CG, Zapol WM. Inhaled nitric oxide, clinical rationale and applications. *Advances in Pharmacology.* Vol. 15: Academic Press; 1995:439-456.
5. Kinsella JP, Neish SR, Ivy DD, Shaffer E, Abman SH. Clinical responses to prolonged treatment of persistent pulmonary hypertension of the newborn with low doses of inhaled nitric oxide [see comments]. *J Pediatr.* 1993;123(1):103-8.
6. Roberts JD, Polaner DM, Lang P, Zapol WM. Inhaled nitric oxide in persistant pulmonary hypertension of the newborn. *Lancet.* 1992;340:818-819.
7. Head CA. Nitric oxide and the lung: an overview. *Artif Organs.* 1997;21(1):5-9.
8. Mupanemunda RH. Current status of inhaled nitric oxide therapy in the perinatal period. *Early Hum Dev.* 1997;47(3):247-62.
9. Ziegler JW, Ivy DD, Kinsella JP, Abman SH. The role of nitric oxide, endothelin, and prostaglandins in the transition of the pulmonary circulation. *Clin Perinatol.* 1995;22(2):387-403.
10. Abman SH, Ivy DD, Ziegler JW, Kinsella JP. Mechanisms of abnormal vasoreactivity in persistent pulmonary hypertension of the newborn infant. *J Perinatol.* 1996;16(2 Pt 2 Su):S18-23.
11. Kinsella JP, Abman SH. Clinical pathophysiology of persistent pulmonary hypertension of the newborn and the role of inhaled nitric oxide therapy. *J Perinatol.* 1996;16(2 Pt 2 Su):S24-7.
12. Kinsella JP, Neish SR, Shaffer E, Abman SH. Low-dose inhalation nitric oxide in persistent pulmonary hypertension of the newborn [see comments]. *Lancet.* 1992;340(8823):819-20.
13. Thadani U, Manyari D, Parker JO, Fung H-L. Tolerance to the circulatory effects of oral isosorbide dinitrate. *Circulation.* 1980;61:526-535.
14. Abrams J. Clinical aspects of nitrate tolerance. *European Heart Journal.* 1991;12(Suppl E):42-52.
15. Thadhani U, Hamilton SF, Olson E, Anderson JL, Kirsten E, Teague SM. Duration of effects and tolerance of slow-release isosorbide-5-mononitrate for angina pectoris. *American Journal of Cardiology.* 1987;59:756-762.
16. Zayek M, Cleveland D, Morin FCd. Treatment of persistent pulmonary hypertension in the newborn lamb by inhaled nitric oxide [see comments]. *J Pediatr.* 1993;122(5 Pt 1):743-50.
17. Zayek M, Wild L, Roberts JD, Morin FCd. Effect of nitric oxide on the survival rate and incidence of lung injury in newborn lambs with persistent pulmonary hypertension. *J Pediatr.* 1993;123(6):947-52.
18. Hyde D. Morphometric and morphologic evaluation of pulmonary lesions in beagle dogs chronically exposed to high ambient levels of air pollutants. *Lab Invest.* 1978;38:455.
19. Hallman M, Waffarn F, Bry K, Bhalla DK, Phalen RF. Surfactant dysfunction after inhalation of nitric oxide. *Amer. Jnl. of Physiol.* 1996;80:2026-34.
20. Rosenberg AA, Kinsella JP, Abman SH. Cerebral hemodynamics and distribution of left ventricular output during inhalation of nitric oxide. *Critical Care Medicine.* 1995;23:1391-1397.
21. Wessel DL, Adatia I, Van Murter LJ, et al. Improved oxygenation in a randomized trial of inhaled nitric oxide for persistent pulmonary hypertension of the newborn. *Pediatrics.* 1997;100(5):7.
22. Mariani B, Barefield ES, W.A.Carlo. The role of nitric oxide in the treatment of neonatal pulmonary hypertension. *Current Opinion in Pediatrics.* 1996;8:118-125.
23. Easa D, Murai DT, Oka B, et al. Early experience with inhaled nitric oxide for the treatment of infants and children with pulmonary hypertension. *Hawaii Medical Journal.* 1996;55:67-69.
24. Kachel W, Varnholt V, Lasch P, Muller W, Lorenz C, Wirth H. High-frequency oscillatory ventilation and nitric oxide: alternative or complementary to ECMO. *Inter Jnl of Arficial Organs.* 1995;18:589-597.
25. Yamazato M, Muraji T, Higashimoto Y, et al. Successful reversal of persistent fetal circulation by inhaled nitric oxide in a newborn with overwhelming sepsis. *Pediatr Surg Int.* 1995;10:381-383.
26. Turbow R, Waffarn F, Yang L, Sills J, Hallman M. Variable oxygenation response to inhaled nitric oxide in severe persistant pulmonary hypertension of the newborn. *Acta Paediatr.* 1995;84:1305-1308.
27. Goldman AP, Tasker RC, Haworth SG, Sigston PE, Macrae DJ. Four patterns of response to inhaled nitric oxide for persistant pulmonary hypertension of the newborn. *Pediatrics.* 1996;98:706-713.
28. Tang SF, Miller O. Low-dose inhaled nitric oxide for neonates with pulmonary hypertension. *J. Paediatr. Child Health.* 1996;32:419-423.

12. References (cont)

29. Skimming JW, Bender KA, Hutchison AA, Drummond WH. Nitric oxide inhalation in infants with respiratory distress syndrome. *J. Pediatr.* 1997;130:225-30.

30. Barefield ES, Karle VA, III JBP, Carlo WA. Inhaled nitric oxide in term infants wi.. hypoxemic respiratory failure. *J Pediatr.* 1996;129:279-286.

31. Muller W, Kachel W, Lasch P, Varnholt V, Konig SA. Inhaled nitric oxide for avoidance of extracorporeal membrane oxygenation in the treatment of severe persistant pulmonary hypertension of the newborn. *Intensive Care Med.* 1996;22:71-76.

32. Day RW, Lynch JM, White KS, Ward RM. Acute response to inhaled nitric oxide in newborns with respiratory failure and pulmonary hypertension. *Pediatrics.* 1996;98:698-705.

33. Muller W, Kachel W, Lasch P, Varnholt V, Konig S. Inhaled nitric oxide during extracorporeal membrane oxygenation for the treatment of severe persistant pulmonary hypertension of the newborn. *Artif Organs.* 1995;20:60-63.

34. Finer NN, Etches PC, Kamstra B, Tierney AJ, Peliowski A, Ryan CA. Inhaled nitric oxide in infants referred for extracorporeal membrane oxygenation: dose response. *J Pediatr.* 1994;124:302-308.

35. Lonnqvist PA, Winberg P, Lundell B, Sellden G, Olsson GL. Inhaled nitric oxide in neonates and children with pulmonary hypertension. *Acta Paedeatrica.* 1994;83:1132-1136.

36. Hoffman GM, Ross RA, E.Day S, R.B.Rice, L.D.Nelin. Inhaled nitric oxide reduces the utilization of extracorporeal membrane oxygenation in persistant pulmonary hypertension of the newborn. *Crit Care Med.* 1997;25:352-359.

37. Francoise M, Gouyon JB, Mercier JC. Hemodynamics and oxygenation changes induced by the discontinuation of low-dose inhalational nitric oxide in newborn infants. *Intensive Care Med.* 1996;22:477-481.

38. Roze J-C, Storme L, Zupan V, Morville P, Mercier J-C. Echocardiographic investigation of inhaled nitric oxide in newborn babies with severe hypoxemia. *Lancet.* 1994;344:303-304.

39. Takata M, Miyasaka K, Sakai H, Fujiwara H, Ito Y, Kawano T. Inhaled nitric oxide and extracorporeal membrane oxygenation in persistant pulmonary hypertension of the newborn. *Acta Paediatrica Japonica.* 1995;37:171-173.

40. Kinsella JP, Schmidt JM, Abman SH. Inhaled nitric oxide treatment for stabilization and emergency medical transport of critically ill newborns and infants. *Pediatrics.* 1995;95:773-776.

41. Abman SH, Kinsella JP, Schaffer MS, Walkening RB. Inhaled nitric oxide in the management of a premature newborn with severe respiratory distress and pulmonary hypertension. *Pediatrics.* 1993;92:606-609.

42. Buhrer C, Merker G, Falke K, Versmold H, Obladen M. Dose-response to inhaled nitric oxide in acute hypoxemic respiratory failure of newborn infants: a preliminary report. *Pediatric Pulomonology.* 1995;19:291-298.

43. Peliowski A, Finer NN, Etches PC, Tierney AJ, Ryan CA. Inhaled nitric oxide for premature infants after prolonged rupture of the membranes. *Journal of Pediatrics.* 1995;126:450-453.

44. Atz AM, Adatia I, Wessel DL. Rebound pulmonary hypertension after inhalation of nitric oxide. *Ann. Thorac. Surg.* 1996;62:1759-64.

45. Atz AM, Adatia I, Jonas RA, Wessel DL. Inhaled nitric oxide in children with pulmonary hypertension and congenital mitral stenosis. *Amer. Jnl. of Cardiology.* 1996;77:316-319.

46. Curran RD, Mavroudis C, Backer CL, M.Sautel, V.R.Zales, Wessel DL. Inhaled nitric oxide for children with congenital heart disease and pulmonary hypertension. *Ann Thorac Surg.* 1995;60:1765-71.

47. Day RW, Guarin M, Lynch JM, Vernon DD, Dean JM. Inhaled nitric oxide in children with severe lung disease: results of acute and prolonged therapy with two concentrations. *Crit Care Med.* 1996;24:215-221.

48. Steinhorn RH, Cox PN, Fineman JR, et al. Inhaled nitric oxide enhances oxygenation but not survival in infants with alveolar capillary dysplasia. *J Pediatr.* 1997;130:417-422.

49. Parker TA, Ivy DD, Kinsella JP, et al. Combined therapy with inhaled nitric oxide and intravenous prostacyclin in an infant with alveolar-capillary dysplasia. *Am J Respir Crit Care Med.* 1997;155(2):743-6.

50. Karamanoukian HL, Gluck PL, Zayek M, et al. Inhaled nitric oxide in congenital hypoplasia of the lungs due to diaphragmatic hernia or oligohydramnios. *Pediatrics.* 1994;94:715-718.

51. Shah N, Jacob T, Exler R, Simmons R, Motoyama E, Nakayama D. Inhaled nitric oxide in congenital diaphragmatic hernia. *Journal of Pediatric Surgery.* 1994;29:1010-1015.

52. Puybasset L, Rouby JJ, Mourgeon E, et al. Inhaled nitric oxide in acute respiratory failure: dose-response curves. *Intensive Care Med.* 1994;20:319-327.

53. Abman SH, Griebel JL, Parker DK, Schmidt JM, Swanton D, Kinsella JP. Acute effects of inhaled nitric oxide in children with severe hypoxemic respiratory failure. *J Pediatr.* 1994;124(6):881-8.

54. Fineman JR, Zwass MS. Inhaled nitric oxide therapy for persistent pulmonary hypertension of the newborn. *Acta Paediatrica Japonica.* 1995;37:425-430.

55. Cioffi WG, Ogura H. Inhaled nitric oxide in acute lung disease. *New Horizons.* 1995;3:73-85.

56. Abman SH, Kinsella JP. Inhaled nitric oxide therapy of pulmonary hypertension and respiratory failure in premature and term neonates. *Adv Pharmacol.* 1995;34:457-74.

12. References (cont)

57. Wessel DL, Adatia I, Thompson JE, Hickey PR. Delivery and monitoring of inhaled nitric oxide in patients with pulmonary hypertension. *Critical Care Medicine*. 1994;22:930-938.
58. Group UCET. UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation. *Lancet*. 1996;348:75-82.
59. O'Rourke PP, Crane RK, Vincenti JP. Extracorporeal membrane oxygenation and conventional medical therapy in neonates with persistant pulmonary hypertension of the newborn: a prospective randomised trial. *Pediatrics*. 1989;84:957-963.
60. Bifano EM, Hankanson DO, Hingre RV, Gross SI. Prospective randomised controlled trial of conventional treatment or transport for ECMO in infants with persistant pulmonary hypertension. *Pediatric Research*. 1992;31:196A.
61. Packer M, Meller J, Medina N, Gorlin R, Herman MV. Rebound hemodynamic events after abrupt withdrawal of nitroprusside in patients with severe chronic heart failure. *NEJM*. 1979;301:1193-1197.
62. Barefield ES, Karle VA, Philips JB, Carlo WA. Randomized, controlled trial of inhaled nitric oxide in ECMO candidates. *Pediatric Research*. 1995;37:195.
63. Assreuy J, Cunha FQ, Liew FY, Moncada S. Feedback inhibition of nitric oxide synthase activity by nitric oxide. *British Journal of Pharmacology*. 1993;108:833-837.
64. Semigran MJ, Cockrill BA, Kacmarek R, Zapol WM, Fifer MA. Hemodynamic effects of inhaled nitric oxide in heart failure. *JACC*. 1994;24:982-988.
65. Hogman M, Frostell C, Arnberg H, Hedenstierna G. Bleeding time prolongation and NO inhalation. *Lancet*. 1993;341:1644-1665.
66. Albert J, Wallen H, Brojiersen A, Frostell C, Hjemdahl P. Effects of inhaled NO on platelet function in vivo in healthy volunteers. *FASEB Journal*. 1995;9:A30 (Abstract).
67. Hayes AW. Principles and Methods of Toxicology. In: Hayes AW, ed. Third ed. New York: Raven Press:50.
68. Wagner HM. Absorption von NO und NO2 in MIK- and MAK-konzentrationen bei der inhalation. *Stuab-Reinhalt. Luft*. 1970;30:380-381.
69. Curran RD, Mavroudis C, Backer CL, Sautel M, Zales VR, Wessel DL. Inhaled nitric oxide for children with congenital heart disease and pulmonary hypertension. *Ann Thorac Surg*. 1995;60:1765-71.
70. Nakajima T, Oda H, Kusumoto S, Nogami H. *Biological effects of nitrogen dioxide and nitric oxide* Ann Arbor: Ann Arbor Science; 1980 Nitrogen Oxides and their effects on health).
71. Muller B, Schafer H, Barth P, Wichert Pv. Lung surfactant components in bronchoalveolar lavage after inhalation of NO2 as markers of altered surfactant metabolism. *Lung*. 1994;172:61-72.
72. Evans MJ, Stephens RJ, Cabral LJ, Freeman G. Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. *American Review of Respiratory Disease*. 1972;143:522-577.
73. Stavert DM, Lehnert BE. Nitric oxide and nitrogen dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods. *Inhalaltion Toxicology*. 1990;2:53-67.
74. Bylin G, Hedenstierna G, Lindvall T, Sundin B. Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. *European Respiratory Journal*. 1988;1:606-612.
75. Hogman M, Frostell C, Hedenstrom H, Hedenstierna G. Inhalation of nitric oxide modulates adult human bronchil tone. *American Review of Respiratory Disease*. 1993;148:1474-1478.
76. NIOSH. NIOSH recommendations for occupational safety and health standards. *Morbidity and mortality weekly report*. 1988;37(Supplement):21.
77. Beckman JS, Bechman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxy-nitrite: implications for endothelial injry from nitric oxide and superoxide. *PNAS*. 1991;288:481-487.
78. Stolar CJ, Snedecor SM, Bartlett RH. Extracorporeal membrane oxygenation and neonatal respiratory failure: experience from the extracorporeal life support organization. *Journal of Pediatric Surgery*. 1991;26:563-571.
79. Milerad J, Walsh WF. Commentary on neonatal ECMO: a North American and Scandanavian perspective. *Acta Paedeatrica*. 1995;84:841-847.
80. Abman SH, Kinsella JP, Schaffer MS, Wilkening RB. Inhaled nitric oxide in the management of a premature newborn with severe respiratory distress and pulmonary hypertension [see comments]. *Pediatrics*. 1993;92(4):606-9.
81. Petros AJ. Down-regulation of endogenous nitric oxide production after prolonged administration. *Lancet*. 1994;344:191.
82. Christou H, Adatia I, Marter LJV, Kourembanas S. Effect of inhaled nitric oxide on endothelin-1 and cyclic guanosine 5'-monophosphate plasma concentrations in newborn infants with persistent pulmonary hypertension. *J. Pediatr*. 1997;130:603-611.

13. Appendix One: list of abbreviations used in the review

a/AO_2: arterial-alveolar oxygen ratio

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

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

ECMO: extracorporeal membrane oxygenation

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

HFJV: high-frequency jet ventilation

HFOV: high-frequency oscillatory ventilation

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

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

MetHb: Methemoglobin (in %).

mmHg: pressure in millimeters of mercury.

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

P_AW: mean airway pressure.

PEEP: positive end-expiratory pressure

PIP: positive inspiratory pressure

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

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

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

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

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

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

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

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

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

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

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

cc: ORIG: NDA 20-845
HFD-110 Division File
HFD-110/CSO Zelda McDonald
HFD-110/Medical Officer Douglas Throckmorton
HFD-110/Secondary Reviewer Norman Stockbridge

NOV - 5 1999

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

Secondary Review

NDA: 20-845 INOmax (nitric oxide inhaled)

Sponsor: INO Therapeutics

Submission: NDA resubmission, dated 25 May 1999.

Review date: November 5, 1999

Reviewer: N. Stockbridge, M.D., Ph.D., HFD-110

/S/

Summary: The original NDA was withdrawn prior to a regulatory decision. The resubmission includes long-term safety data from earlier studies and results of a third well-controlled study.

Distribution: NDA 20-845
HFD-110/Project Manager
HFD-110/Stockbridge
HFD-110/Throckmorton
HFD-710/Cui

Table of contents

1 Introduction

NDA 20-845 was submitted in June 1997 with two pivotal studies and two supportive studies. The submission was given a priority review, and the review team was on-track for meeting the goal date when the application was withdrawn by the sponsor. The resubmission was received 26 May 1999 and it has a 6-month goal date of 26 November 1999.

There are no outstanding issues with regard to chemistry and manufacturing, DSI, or pharmacology.

Dr. Nguyen's Biopharmaceutics (undated draft) review raises the issue that pharmacokinetic data for nitric oxide comes from studies in adults. This problem is addressed in the marked-up label.

This secondary review is based upon the clinical review of Dr. Throckmorton (dated 29 October 1999) and the statistical review of Dr. Cui (dated 3 November 1999). The primary clinical review, in particular, contains much information about the evolution of concerns through a complex development program. The goal of this review is to summarize the current state of knowledge and to present the options for regulatory action.

2 Summary of pivotal studies

The studies forming the basis for decision-making are summarized in the sections below.

2.1 NINOS

NINOS was a NICHD-sponsored, multi-center, multi-national, randomized, double-blind, parallel, placebo-controlled study undertaken in term and near-term infants with hypoxic respiratory failure (meconium aspiration, sepsis or pneumonia, respiratory distress syndrome, or primary pulmonary hypertension of the newborn) and no serious structural cardiac abnormalities. Subjects were randomized to placebo (100% O_2) or ~95%O_2 plus 20 ppm nitric oxide in nitrogen. Non-responders (assessed by PaO_2) could be up-titrated to 80 ppm after 30 minutes and 30 minutes later could be considered treatment failures. Responders underwent weaning attempts beginning at 2 hours. The maximum protocol-permitted duration of treatment was 14 days. Other conventional treatments were allowed, including surfactant, vasodilators, and high-frequency ventilation. The primary end point was mortality or extracorporeal membrane oxygenation (ECMO) prior to the earlier of hospital discharge or 120 days. Follow-up for neurological status was planned for 18 to 24 months. By design, NINOS collected safety data pertaining to only a limited set of events.

NINOS was stopped early (but with 235 of 250 planned subjects enrolled), because of apparent benefit of active treatment on the composite end point of mortality or ECMO. Placebo and INO treatment groups were similar. About 50% had meconium aspiration, 20% had sepsis or pneumonia, and 20% had idiopathic primary pulmonary hypertension (PPHN). Interpretation of the NINOS trial was complicated by numerous protocol violations, the worst of which was wrong treatment given (7 subjects randomized to placebo and 1 subject randomized to INO; events spread among multiple centers), and also by discrepancies between meeting ECMO criteria and actual ECMO. These analyses are summarized in Table 1.

Table 1. Selected analyses (NINOS).

	Randomized treatment			Actual treatment		
	Placebo N=121	INO N=114	P	Placebo N=112	INO N=118	P
Death	17%	14%	0.6	15%	14%	0.87
ECMO	55%	39%	0.014	55%	41%	0.026
Met ECMO criteria	69%	59%	0.12	—	—	—
Combined (primary)	64%	46%	0.006	63%	47%	0.015

Most clearly, INO improved acute oxygenation, as assessed by PaO_2 (10 vs. 58 mmHg, $p<0.001$), change in oxygenation index (OI; +0.8 vs. -14, $p<0.001$), or change in A-aDO_2 (-7 vs. -60 mmHg, $p<0.001$). Most subjects (~80%) who met criteria for ECMO, in both treatment arms, did so by meeting oxygenation criteria.

Point estimates of relative risk by etiology ranged from 0.4 for PPHN to 1.1 for RDS; the confidence limits neither guarantee homogeneity nor refute it.

About 74% of NINOS subjects had 18- to 24-month follow-up data, during which time there was only one death. Hospitalizations and utilization of nursing or other special services was similar in the treatment groups, as were various indicators of long-term pulmonary disease (need for oxygen, ventilator, bronchodilators). The groups were not distinguishable with regard to neurological exams or assessments (mental development index, psychomotor development index, or behavioral rating scale).

2.2 INO-01/-02

Studies INO-01 and INO-02 were the (original) sponsor's trials, which, through a series of amendments became a single trial with reduced recruitment goals. Upon publication of the NINOS findings, it failed to meet even those goals; it was stopped with about 50% of its final targeted enrollment. This multi-center, randomized, double-blind, parallel, placebo-controlled study enrolled only term and near-term infants with PPHN. Subjects were randomized to placebo or INO 5, 20, or 80 ppm, for up to 14 days or until there was clinical improvement (PaO_2 > 60 mmHg or FiO_2 <60%). While receiving study gas, surfactant and high-frequency ventilation were prohibited. The primary end point was the incidence at 28 days of death, ECMO, intraventricular hemorrhage, brain infarct, seizures, or bronchopulmonary dysplasia.

Treatment groups in the INO-01/-02 study appeared to be similar at baseline. By OI, they were somewhat less sick on average than subjects in the NINOS study, probably because investigators wanted to use surfactant or high-frequency ventilation in sicker infants. Results for the primary end point and the components thereof are shown in Table 2.

Table 2. End point analyses for INO-01/-02.

	Placebo N=41	INO 5 ppm N=41	INO 20 ppm N=36	INO 80 ppm N=37	P
Death	2%	5%	11%	8%	0.44
ECMO	34%	24%	25%	16%	0.34
Neurological sequelae	26%	15%	34%	23%	0.35
Bronchopulmonary dysplasia	13%	24%	10%	9%	0.23
Death or ECMO	39%	28%	39%	22%	0.25
Combined (primary)	56%	50%	60%	39%	0.34

There were trends, at least, for improvements in oxygenation (increase in PAO_2, decrease in OI), acutely, but no trend apparent in indications of clinical benefit—days on oxygen, days on ventilation, or days in hospital. Peak NO_2 and methemoglobin levels were similar in placebo and INO groups of 5 and 20 ppm, but were elevated in the 80 ppm group. At some time during study, about 20% of subjects on 80 ppm exceeded the 3 ppm limit for NO_2 and about 35% exceeded the 7% limit on methemoglobin[1].

Long-term outcome was reported for about 93% of the group. For another 3%, there was only telephone follow-up. There was only one reported death in this period (placebo), but the relatively large number of losses to follow-up could conceal a substantial effect. More subjects from INO groups (29%) than from placebo (17%) were receiving special medical program care (physical therapy, infant stimulation, etc.) at 1 year. The groups were not distinguishable with regard to neurological or audiological exams or assessments (mental development index, or psychomotor development index). However, seizures were more common in the INO groups (7% vs. 0%). Thirteen percent of the INO subjects vs. none of the placebo group were on supplemental oxygen at 1 year, but there were no apparent differences in the rates of asthma, bronchiolitis, bronchitis, or pneumonia. INO subjects were, however, more likely to be on respiratory medications (15% vs. 6%).

2.3 CINRGI

CINRGI was an investigator-initiated, multi-center, randomized, double-blind, parallel, placebo-controlled study of term or near-term newborns with PPHN. Structural heart disease was an exclusion, but use of high-frequency ventilation was encouraged where appropriate. Subjects on INO received 20 ppm for 4 to 24 hours. The dose was reduced to 5 ppm for PaO_2 >60 mmHg, and this dose was maintained until FIO_2 was <0.7, day 4, or age 7 days. INO could be restarted as needed during the treatment period. The primary end point was ECMO.

CINRGI 'completed' with enrollment at 186 of 206 planned. Treatment groups were similar with regard to demographics, baseline disease, and concomitant drug use, with the following exceptions. Air-leak syndrome was more common at baseline in the placebo group (25% vs. 11%). PaO_2 (78 vs. 54 mmHg) and SaO_2 (90 vs. 84%) were higher and OI was lower (35 vs. 44) in the INO group. These imbalances in oxygenation may be the result of baseline measurements being made after the initiation of study gas (since these operations were performed by different personnel); the imbalances are much less

[1] *The limits on NO_2 reflect concerns about its toxicological effects in the lung (manifested by impairment of CO_2 exchange). It is much less clear how to set the limits for methemoglobinemia, since the effect is merely to reduce the oxygen-carrying capacity of the blood. The reduction in carrying capacity might be offset by improvements in ventilation-perfusion matching; the way to tell is to measure oxygen delivery, but this does not seem to have been done in this or other studies.*

evident in assessments made 2 or 4 hours prior to 'baseline'. One subject randomized to placebo actually received INO.

There were two early deaths among subjects randomized to INO and one among subjects randomized to placebo. ECMO was more common among subjects randomized to placebo (57% vs. 31%; p=0.001); this result by ITT was much the same as the one by actual treatment, 'per protocol', or after adjustment for baseline imbalance in OI or other nominally imbalanced group characteristics.

Mortality at 28 days was 6% on placebo and 3% on INO. The duration of hospitalization was similar in the two groups.

Chronic lung disease was more common on placebo than on INO (13% vs. 3%), and it did not correlate with baseline presence of air-leak. Use of home oxygen or pulmonary medications at discharge were also somewhat more common in the placebo group.

PaO_2, assessed as either absolute values or as changes from (dissimilar) baselines, was consistently greater in the INO treatment group at times up to 24 hours, when analyzed for subjects remaining in the study.

Double-differences (baseline and placebo) in PaO_2, but not oxygenation index, diminished during the first 24 hours, according to a LOCF analysis described by Dr. Cui. Plausibly, the effects of INO diminish over time, much as do the effects of organic nitrate nitric oxide donors. On the other hand, the placebo group may have benefited from more aggressive later concomitant therapy.

The treatment effect on ECMO was similar in subjects with meconium aspiration, pneumonia or sepsis, respiratory distress syndrome, or persistent pulmonary hypertension.

Twenty-six subjects with lung hypoplasia attributed to congenital diaphragmatic hernia were randomized and treated, but were not, by protocol, included in the above assessments. Overall mortality was about 40%. There was evidence of neither benefit nor harm in this small subgroup.

About 1/3 of randomized subjects had 6-month and about 1/4 had 12-month follow-up data. The trend was for less need for oxygen and pulmonary medications in the INO group, but the differences (and samples) are small.

3 Conclusions and options for regulatory action

Each of the three trials has been described as double-blind, but probably none of them were effectively blinded. Protocols called for unblinded personnel to manage the ventilation of each subject. At best, the tanks and valves were cloaked. In addition to the mechanics of blinding, there was the obvious 'problem' that nitric oxide acutely improves oxygenation and was associated with elevations in NO_2 and methemoglobin.

Dr.Cui raises the issue that knowledge of treatment could have influenced decisions about when to implement ECMO; he suggests that 'meeting ECMO criteria' would have been more objective. Certainly, from the NINOS study in which 'met ECMO criteria' was analyzed, there is a much weaker indication of benefit.

Avoidance of ECMO is a legitimate clinical benefit. While not associated with the degree of mortal and morbid risks it once was, the process remains risky and it is not universally available.

Nitric oxide use was associated with less use of ECMO in two studies. In the third, where the results lean appropriately, enrollment was only half of that planned, subjects were less sick, and investigators were constrained from using surfactant and high-frequency ventilation.

What nitric oxide most clearly does is produce an acute improvement in oxygenation, plausibly because the gas is delivered preferentially to well ventilated alveoli and produces vasodilation predominantly there. Where criteria for ECMO were defined, oxygenation was the principal determinant of who went for ECMO.

Various studies in the literature indicate that doses of nitric oxide above those studied in any of these trials are associated with progressively greater vasodilation, but even 80 ppm appears to confer no further clinical benefit, plausibly because of elevations in NO_2 exposure and increases in methemoglobin. In a clinical database so small, it is sufficiently difficult to conclude there is net clinical benefit to any dose, much less to conclude when the dose-net benefit curve seems to have peaked. The data are such that the label should suggest use similar to that in the most compelling study, CINRGI, and then neither encourage nor enjoin use above 20 ppm. Likewise, the only advice to give regarding the appropriate duration of treatment comes from the clinical studies.

Use of nitric oxide had effects on mortality too small to see in a few hundred subjects. Earlier studies raised some concerns about development of chronic lung injury manifesting as asthma, brochopulmonary dysplasia, or prolonged reliance upon supplemental oxygen. These concerns seem largely unsupported by CINRGI and long-term follow-up data from the other studies.

Nitric oxide appears to have little impact, favorable or adverse, on the duration of hospitalization or long-term neurological sequelae.

The regulatory options are two.

This application might not be approved, because of inadequacies in the design and implementation of the pivotal studies and the lack of demonstrated long-term benefit—decreased mortality, decreased hospitalization, or improved pulmonary or neurological status. However, in exercising this option, one must recognize that one would not likely create an environment in which a better placebo-controlled study would be forthcoming.

The other option is to approve the use of nitric oxide with a label suitably circumspect with regard to the potential benefits and risks. Some three hundred neonatologists and anesthesiologists have already indicated a substantial willingness to operate with greater uncertainty. A draft approval letter and marked-up label accompany the package.