ADME NTP Study S0039 C.I. Vat blue 1

The contractor used the common term indigo for the test article.
Sex/Species: adult male F344 rats.
Vehicles: intravenous, dimethyl sulfoxide (DMSO):F344 rat serum (1.45:9 v,v); oral, corn oil; dermal, ointment base or dry powder.

CASRN 482-89-3

Radiolabeled with carbon-14 uniformly in the phenyl moieties; CI Vat Blue #1, [Phenyl-U-^{14}C]-

Studies Performed:

1. Single 0.2 mg/kg intravenous dose to rats with sacrifice at 0.25, 0.75, 2.0, 6.0, 24, 72, or 240 hours postdose.

2. Single 2.97, 31.1, or 305 mg/kg oral gavage dose to rats with sacrifice 96 hours postdose.

3. Single 3.38 mg/kg oral gavage dose to rats with sacrifice 72 hours postdose (purified [^{14}C]indigo).

4. Single 1.28 mg/kg dermal dose to rats with covered dose site and sacrifice 192 hours postdose (0.42 mg/cm^2; vehicle, dry powder).

5. Single 3.70 mg/kg dermal dose to rats with covered dose site and sacrifice 192 hours postdose (1.19 mg/cm^2; vehicle, ointment base).

The [^{14}C]indigo used was circa 70% radiochemically pure as analyzed by HPLC. In-house cocrystallization with sublimed unlabeled indigo resulted in a radiochemical purity of 91% (product referred to as “purified”) which was administered orally to rats in corn oil at 3.38 mg/kg (72 hour sacrifice). Unlabeled indigo appeared essentially pure by HPLC analysis.

For the 3.7 mg/kg dermal and the 2.97-305 mg/kg oral doses (96 hour sacrifice), the 70% pure radiolabeled indigo was dried *in vacuo* and then reconstituted for individual doses. For the 1.28 mg/kg dermal dose, the dried radiolabeled indigo was applied directly to the dose site. For the 3.7 mg/kg dermal route, 2 mg of dried [^{14}C]indigo was mixed with 28 mg of the ointment base (w/w). The ointment base was 9 g of PEG 1540, 6 g PEG 400, and 3 g propylene glycol that were melted together.

The amount of indigo absorbed from the gastrointestinal tract was calculated by comparing the 0-96 hour excretion of ^{14}C in urine following an oral dose to that following the 0.2 mg/kg intravenous dose (Table 8). The amount of indigo absorbed through the

skin was calculated by comparing the 0-72 hour excretion of ^{14}C in urine after a dermal dose to that following the 0.2 mg/kg intravenous dose (Table 9).

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Table 1

Cumulative Excretion of Total ^{14}C After Intravenous Administration of 0.2 mg/kg of [^{14}C]Indigo (% Dose)[a]

Time (hr)	72 hr Experiment[b]				Time (hr)	240 hr Experiment[d]		
	Urine	Feces	Breath	Total		Urine	Feces	Total
2	1.05 ± 1.61			1.05 ± 1.61	24	19.0 ± 3.8	19.9 ± 4.9	38.9 ± 3.0
4	5.94 ± 2.77			5.94 ± 2.77	48	23.8 ± 4.1	30.7 ± 0.9	54.5 ± 4.3
6	6.22 ± 2.95			6.22 ± 2.95	72	26.6 ± 4.2	35.4 ± 0.8	62.3 ± 3.9
8	8.86 ± 1.43	1.36 ± 2.21		10.2 ± 1.4	96	28.2 ± 4.2	37.3 ± 1.0	65.5 ± 3.8
24	17.6 ± 2.6	20.0 ± 14.5	0.23 ± 0.00[c]	37.7 ± 13.7	120	29.5 ± 4.4	39.2 ± 0.8	68.7 ± 4.2
28	18.2 ± 3.1			38.3 ± 13.2	144	30.2 ± 4.5	40.1 ± 0.5	70.3 ± 4.3
32	16.4 ± 4.2	33.0 ± 6.4		52.6 ± 5.6	168	31.1 ± 4.5	41.0 ± 0.6	72.2 ± 4.3
48	21.5 ± 2.3	39.6 ± 6.3	0.41 ± 0.03[c]	61.4 ± 6.1	192	31.8 ± 4.3	41.8 ± 0.7	73.5 ± 4.0
56	22.3 ± 2.4			62.1 ± 5.7	216	32.2 ± 4.3	42.2 ± 0.7	74.4 ± 4.1
72	23.5 + 2.7	44.3 ± 8.0	0.49 ± 0.01[c]	68.1 ± 6.1	240	32.4 ± 4.3	42.6 ± 0.7	75.1 ± 4.1
72 + cage wash	25.8 ± 4.1			70.4 ± 4.6	240 + cage wash	34.7 ± 3.4		77.3 ± 3.2

[a] Original report appendix has data from individual rats.

[b] Average of 3 Rats ± S.D.

[c] Average of 2 Rats ± Range

[d] Average of 4 Rats ± S.D.

Table 2

Cumulative Excretion of Total ^{14}C after Oral Administration of Indigo (% Dose)[a]

Dose (mg/kg):	2.97			31.1			305		
Excreta : Time (hr)	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
2	0.00 ± 0.00		0.00 ± 0.00	0.26 ± 0.23		0.26 ± 0.23	0.00 ± 0.00		0.00 ± 0.00
4	0.00 ± 0.00		0.00 ± 0.00	0.28 ± 0.25		0.28 ± 0.25	0.42 ± 0.73		0.42 ± 0.73
6	0.00 ± 0.00		0.00 ± 0.00	0.29 ± 0.25		0.29 ± 0.25	0.51 ± 0.88		0.51 ± 0.88
8	5.19 ± 4.60	3.11 ± 3.38	8.30 ± 1.22	2.63 ± 2.24	0.29 ± 0.50	2.92 ± 2.69	1.08 ± 1.86	0.03 ± 0.05	1.11 ± 1.92
24	10.6 ± 6.58	70.8 ± 8.13	81.4 ± 8.38	7.66 ± 0.77	56.2 ± 8.28	63.8 ± 7.76	7.91 ± 0.34	60.9 ± 8.33	68.9 ± 8.06
28	11.0 ± 6.63	70.8 ± 8.13	81.9 ± 8.09	7.79 ± 0.85	56.2 ± 8.28	63.9 ± 7.68	8.09 ± 0.31	60.9 ± 8.33	69.0 ± 8.11
32	11.0 ± 6.62	76.8 ± 10.4	87.9 ± 7.16	7.86 ± 0.95	65.2 ± 2.74	73.0 ± 3.31	8.23 ± 0.46	66.5 ± 6.05	74.7 ± 6.03
48	11.3 ± 6.81	85.3 ± 5.10	96.4 ± 4.22	8.36 ± 1.04	75.4 ± 1.10	83.7 ± 2.12	8.62 ± 0.17	74.6 ± 2.06	83.2 ± 1.95
56	11.3 ± 6.83	85.3 ± 5.10	96.4 ± 4.21	8.42 ± 1.03	75.4 ± 1.10	83.8 ± 2.11	8.72 ± 0.13	74.6 ± 2.06	83.3 ± 1.96
72	11.4 ± 6.87	87.0 ± 3.95	98.4 + 4.76	8.54 ± 1.03	77.6 ± 2.05	86.1 ± 3.06	8.80 ± 0.07	76.5 ± 1.38	85.3 ± 1.32
96	11.4 ± 6.88	87.7 ± 3.33	98.9 ± 4.75	8.87 ± 1.20	78.2 ± 2.35	87.1 ± 3.53	8.88 ± 0.04	76.8 ± 1.46	85.6 ± 1.46

Table 3

Cumulative Excretion of Total ^{14}C After Oral Administration of 3.38 mg/kg of Purified [^{14}C]Indigo (% Dose)

Time (hr)	Rat I50			Rat I51			Rat I52			Average ± S.D.		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
24	2.37	36.0	38.4	2.27	59.1	61.4	2.98	72.5	75.5	2.54 ± 0.38	55.9 ± 18.5	58.4 ± 18.7
48	3.48	72.5	76.0	2.57	80.1	82.7	3.36	77.8	81.2	3.14 ± 0.49	76.8 + 3.9	79.9 ± 3.52
72	3.68	74.7	78.4	2.67	81.1	83.8	3.46	78.4	81.9	3.27 ± 0.53	78.1 + 3.21	81.4 + 2.74
72 + Cage Wash	3.96		78.7	3.32		84.4	3.72		82.1	3.67 ± 0.32		81.7 ± 2.87

Table 4

Cumulative Excretion of ^{14}C After Dermal Administration of Indigo (% Dose)

Time (hr)	1.28 mg/kg dose as a dry powder[a]			3.70 mg/kg dose in an ointment base[b]		
	Urine	Feces	Total	Urine	Feces	Total
2	0.00 ± 0.00		0.00 ± 0.00			
4	0.00 ± 0.00		0.00 ± 0.00			
6	0.01 ± 0.01		0.01 ± 0.01	0.00 ± 0.00		0.00 ± 0.00
8	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
24	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.03	0.18 ± 0.08	0.03 ± 0.03	0.21 ± 0.10
28	0.05 ± 0.02	0.02 ± 0.01	0.06 ± 0.03	0.19 ± 0.08		0.21 ± 0.11
32	0.05 ± 0.02	0.04 ± 0.03	0.08 ± 0.03	0.23 ± 0.05	0.03 ± 0.02	0.26 ± 0.06
48	0.07 ± 0.03	0.04 ± 0.01	0.10 ± 0.03	0.31 ± 0.03	0.05 ± 0.04	0.36 ± 0.05
56	0.07 ± 0.03	0.04 ± 0.01	0.11 ± 0.03	0.32 ± 0.05		0.37 ± 0.07
72	0.08 ± 0.03	0.05 ± 0.01	0.13 ± 0.03	0.38 ± 0.08	0.08 ± 0.06	0.46 ± 0.08
96	0.09 ± 0.03	0.07 ± 0.01	0.16 ± 0.03	0.45 ± 0.12	0.11 ± 0.08	0.56 ± 0.12
120	0.10 ± 0.03	0.09 ± 0.01	0.18 ± 0.03	0.48 ± 0.12	0.15 ± 0.09	0.63 ± 0.11
144	0.10 ± 0.03	0.10 ± 0.00	0.20 ± 0.03	0.51 ± 0.11	0.17 ± 0.10	0.68 ± 0.10
168	0.10 ± 0.04	0.11 ± 0.01	0.21 ± 0.04	0.53 ± 0.12	0.19 ± 0.11	0.71 ± 0.10
192	0.14 ± 0.01[c]	0.12 ± 0.00[c]	0.26 ± 0.01[c]	0.53 ± 0.12	0.20 ± 0.12	0.73 ± 0.10
192 + Cage Wash	0.18 ± 0.02[c]		0.30 ± 0.02[c]	0.60 ± 0.11		0.80 ± 0.10

[a] Average for 3 Rats ± S.D. Average dose for 3 rats is 1.51 mg/kg. Original report appendix has data from individual rats.

[b] Average for 3 Rats ± S.D. Original report appendix has data from individual rats.

[c] Average for 2 Rats ± Range. Average dose for the 2 rats is 1.28 mg/kg.

Table 5

Cumulative Biliary Excretion of ^{14}C After an Intravenous Dose of 0.22 mg/kg of [^{14}C]Indigo (% Dose)

End of Collection Period (hr)	Rat 47	Rat 48	Rat 49	Average ± S.D.
0.08	0.20	0.17	0.16	0.18 ± 0.02[b]
0.17	0.95	---[a]	1.81	1.38 ± 0.43[b]
0.25	1.72	1.45	3.49	2.22 ± 1.11
0.33	2.55	2.83	5.00	3.46 ± 1.34
0.50	3.84	4.17	7.32	5.11 ± 1.92
0.75	5.64	5.06	9.79	6.83 ± 2.58
1.00	7.25		11.5	
1.25	8.42			
1.5			14.0	
2.0			15.8	
3.0			18.3	
4.0			20.2	
5.0			21.8	
6.0			23.2	
7.0			24.6	

[a]Not enough sample to analyze.

[b]Average of 2 Rats ± Range.

Table 6

Excretion of Unmetabolized Indigo in Bile and Urine after Intravenous Administration of 0.22 mg/kg of [^{14}C]Indigo

	Collection Period (hr)	Rat No.	Amount Excreted as Indigo (% Dose)	Amount Excreted % of Total Excreted ^{14}C Accounted for by Indigo
Bile	0-0.08	I47	0.0023	1.15
	0-0.08	I48	0.0024	1.41
	0-0.08	I49	0.0018	1.13
	0.17-0.25	I47	0.0064	0.83
	0.17-0.25	I48	0.0098	0.77
	0.17-0.25	I49	0.014	0.83
	5.0-6.0	I45	0.0056	0.39
Urine	0-4.0	I2	0.0067	0.08
	0-4.0	I3	0.0053	0.08
	0-4.0	I28	0.0034	0.12
	0-24	I29A	0.009	0.06
	0-24	I30	0.012	0.07
	0-24	I31	0.026	0.11
	0-24	I32	0.016	0.08
	48-72	I29A	0.002	0.08
	48-72	I30	0.0035	0.13
	48-72	I31	0.0037	0.13
	48-72	I32	0.0031	0.10
	216-240	I29A	0.0009	0.41
	216-240	I30	0.0017	0.41
	216-240	I31	0.0017	0.49
	216-240	I32	0.0011	0.39

Table 7

Recovery of Radioactivity after Administration of Indigo to Rats (% Dose[a])

Time (hr)	Route	Dose (mg/kg)	Urine	Feces	Breath	Selected Tissues	Tail	Unabsorbed Dose[d]	Cage Washes	Carcass	Total
0.25	IV	0.22				72.7 ± 6.3	3.20 ± 3.62			11.1 ± 1.0	87.1 ± 3.0
0.75	IV	0.22				64.9 ± 3.3	1.85 ± 0.26			14.3 ± 3.78	81.1 ± 2.2
2.0	IV	0.22				53.3 ± 5.5	5.19 ± 4.19			14.1 ± 3.74	72.6 ± 2.3
6.0	IV	0.23	11.2 ± 2.8	0.83 ± 1.4		62.4 ± 2.4	2.28 ± 2.96			9.20 ± 2.21	85.9 ± 3.2
24	IV	0.23	22.7 ± 4.8	24.9 ± 1.9		31.0 ± 1.9	1.61 ± 1.46			5.55 ± 1.24	85.8 ± 6.6
72	IV	0.22	23.5 ± 2.70	44.3 ± 8.0	0.50 ± 0.02[c]	14.3 ± 0.2	1.57 ± 1.39		2.30 ± 1.6	2.61 ± 0.51	88.8 ± 5.1
240	IV	0.24	32.4 ± 4.3[b]	42.6 ± 0.7[b]		4.43 ± 0.35[b]	3.64 ± 2.38[b]		2.24 ± 0.98[b]	1.30 ± 0.10[b]	86.6 ± 2.6[b]
72	Oral	3.38	3.27 ± 0.53	78.1 ± 3.2					0.40 ± 0.22		81.7 ± 2.9
96	Oral	2.97	11.4 ± 6.9	87.7 ± 3.3	0.01 ± 0.01	0.16 ± 0.04			0.74 ± 0.40	0.28 ± 0.07	100.2 ± 7.6
96	Oral	31.1	8.60 ± 1.02	78.2 ± 2.4		0.11 ± 0.01			1.00 ± 0.47	0.20 ± 0.05	88.1 ± 3.1
96	Oral	3.05	8.88 ± 0.04	76.8 ± 1.46		0.08 ± 0.01			0.29 ± 0.10	0.13 ± 0.03	86.2 ± 1.5
192	Dermal	3.70[e]	0.55 ± 0.13	0.20 ± 0.13		0.00		91.5 ± 4.2	0.07 ± 0.01	0.04 ± 0.00	92.4 ± 4.2
192	Dermal	1.28[c,f]	0.14 ± 0.02[c]	0.12 ± 0.00		0.00		74.9 ± 3.4	0.04 ± 0.03[c]	0.03 ± 0.00[c]	75.2 ± 3.4[c]

[a] Average of 3 rats ± S.D.
[b] Average of 4 rats ± S.D.
[c] Average of 2 rats ± range. (The third animal contaminated his cage by removing his patch.)
[d] From topical application; recovered from covering and epidermis.
[e] Dose in mg/cm^2 = 1.18.
[f] Dose in mg/cm^2 = 0.42.

Table 8

Calculated Absorption of Oral Doses of [^{14}C]Indigo[a]

Dose (mg/kg)	% Dose Absorbed ± SD	Amount Absorbed (mg/kg)
3.0	58 ± 4[b]	1.7
31	34 ± 6	10.5
305	33 ± 0.6	100

[a]Based on urinary excretion (0-96 hr)
[b]Range (2 animals only)

Table 9

Calculated Absorption of Dermal Doses of [^{14}C]Indigo[a]

Dose mg/cm^2	% Dose Absorbed	Amount Absorbed (mg/cm^2)
1.18 (in ointment)	2	0.03
0.42 (dry powder)	0.7	0.003

[a]Based on urinary excretion. The reference period used from the IV study was 0-72 hr.

Table 10

Concentration of Total ^{14}C in Blood and Plasma after Administration of [^{14}C]Indigo[a]

Time (hr)	Dose (mg/kg)	Route	Plasma ng-eq/g	Blood ng-eq/g	Plasma Dose/g	Blood Dose/g
0.25	0.2	IV	160 ± 70	130 ± 30	0.16 ± 0.04	0.16 ± 0.04
0.75	0.2	IV	160 ± 50	120 ± 20	0.22 ± 0.08	0.16 ± 0.03
2.0	0.2	IV	100 ± 30	76 ± 8	0.14 ± 0.04	0.10 ± 0.01
6.0	0.2	IV	50 ± 6	43 ± 3	0.06 ± 0.00	0.05 ± 0.00
24.0	0.2	IV	19 ± 1	21 ± 3	0.02 ± 0.00	0.03 ± 0.00
72.0	0.2	IV	7.7 ± 0.6	9.5 ± 0.6	0.01 ± 0.00	0.01 ± 0.00
240.0	0.2	IV	1.6 ± 0.2[b]	3.2 ± 0.2[b]	0.002 ± 0.000[b]	0.004 ± 0.000[b]
96.0	3.0	Oral	11 ± 5	20 ± 5	0.001 ± 0.000	0.002 ± 0.001
96.0	31	Oral	63 ± 5	150 ± 20	0.0006 ± 0.0000	0.001 ± 0.000
96.0	305	Oral	310 ± 60	1300 ± 60	0.0003 ± 0.0000	0.001 ± 0.000
192.0	1.5	Dermal	1.3[c] ± 2.3	1.8[c] ± 2.3	0.0002[c] ± 0.0003	0.0003[c] ± 0.0003
192.0	3.7	Dermal	0.09[c] ± 0.09	2.8[c] ± 0.4	0.0000[c]	0.0002[c] ± 0.0003

[a]Values are the averages for 3 rats ± S.D.
[b]Values are the averages for 4 rats ± S.D.
[c]These values are not reliable due to the very small amount of ^{14}C in the blood.

Table 11

Concentration of ^{14}C-Labeled Compounds in Tissues after Intravenous Administration of [^{14}C]Indigo (ng-eq/g)[a]

	0.25 hr	0.75 hr	2.0 hr	6.0 hr	24.0 hr	72.0 hr	240.0 hr[d]
Skin (Ears)	26 ± 1	32 ± 6	19 ± 3	16 ± 1	15 ± 3	8.1[b]	4.9 ± 0.9
(Belly)	25 ± 3	27 ± 5	10 ± 9	13 ± 2	7.7 ± 1.2	5.4 ± 0.6	7.6 ± 4.7
(Hindquarters)	16 ± 2	20 ± 4	14 ± 0	16 ± 7	6.8 ± 0.2[c]	5.1 ± 0.6	3.5 ± 0.5
(Back of Neck)	19 ± 1	20 ± 2	15 ± 2	15 ± 8	8.7 ± 3.7	5.0 ± 0.8	3.0 ± 1.3
Esophagus	70 ± 40	33 ± 4	25 ± 10	18 ± 5	22 ± 17	5.6 ± 0.7	
Stomach	93 ± 2	110 ± 80	91 ± 16	24 ± 6	6.6 ± 1.8	5.7 ± 3.6	
Liver	4400 ± 500	4600 ± 100	3900 ± 500	3200 ± 600	1500 ± 200	630 ± 60	180 ± 30
Lungs	3500 ± 1000	2800 ± 800	2500 ± 500	2300 ± 300	2100 ± 500	640 ± 20	700 ± 50
Heart	140 ± 20	100 ± 10	62 ± 6	50 ± 1	24 ± 2	9.8 ± 1.1	
Kidneys	370 ± 10	380 ± 70	190 ± 6	150 ± 6	120 ± 20	65 ± 10	54 ± 5
Adipose (Kidney)	24 ± 8	26 ± 7	18 ± 4	8.5 ± 5.8	7.9 ± 2.7	2.4 ± 0.1	1.1 ± 0.3
(Epididymis)	14 ± 3	18 ± 1	14 ± 2	8.8 ± 0.2	5.6 ± 1.5	3.0 ± 0.9	0.58 ± 0.30
(Mesenteric)	32 ± 9	39 ± 6	22 ± 4	11 ± 3	6.9 ± 1.7	2.6 ± 0.5	1.3 ± 0.4
Adrenals	210 ± 40	150 ± 40	130 ± 30	150 ± 20	91 ± 27	78 ± 72	26 ± 4
Small Intestines	164 ± 67	640 ± 220	460 ± 250	190 ± 90	50 ± 1	28 ± 8	4.4 ± 1.6
Large Intestines	33 ± 3	32 ± 9	21 ± 6	820 ± 660	270 ± 50	92 ± 43	9.2 ± 7.2
Cecum	19 ± 5	17 ± 2	34 ± 21	2300 ± 800	370 ± 100	72 ± 5	
Seminal Vesicles	16 ± 1	17 ± 2	9.0 ± 1.0	5.9 ± 0.8	3.6 ± 0.3	2.8 ± 1.7	
Testes	19 ± 2	22 ± 2	17 ± 3	11 ± 1	6.6 ± 0.6	4.4 ± 0.2	
Prostate	24 ± 6	23 ± 5	13 ± 2	10 ± 5	4.5 ± 0.1	10 ± 6.7	
Muscle (Neck)	31 ± 3	25 ± 3	13 ± 4	8.3 ± 1.8	5.0 ± 1.1	4.0 ± 1.8	1.5 ± 1.0
(Hind Leg)	28 ± 3	25 ± 1	13 ± 1	9.1 ± 2.6	3.9 ± 0.8	2.1 ± 0.6[c]	1.1 ± 0.5
(Abdomen)	22 ± 4	23 ± 2	0.16 ± 0.02	10 ± 3	4.0 ± 0.7	2.5 ± 0.6	1.2 ± 0.7
Brain	62 ± 11	38 ± 6	24 ± 2	14 ± 3	5.5 ± 0.4	1.3 ± 0.5	±
Eyes	15 ± 2	16 ± 1	9.2 ± 0.8	6.0 ± 0.8	3.6 ± 0.4	2.7 ± 1.0	±
Spleen	3200 ± 500	2700 ± 600	2300 ± 200	2700 ± 300	2100 ± 100	860 ± 220	560 ± 60
Plasma	160 ± 70	160 ± 50	100 ± 30	50 ± 6	19 ± 1	7.7 ± 0.6	1.6 ± 0.2
Blood	130 ± 30	120 ± 20	76 ± 8	43 ± 3	21 ± 3	9.5 ± 0.6	3.2 ± 0.2

[a]Values are the averages for 3 rats ± S.D. Original report appendis has data from individual rats.

[b]Data for 1 rat only.

[c]Data for 2 rats only.

[d]Values are the averages for 4 rats ± S.D.

Table 12

Tissue-Blood Ratios of ^{14}C-Labeled Compounds after Intravenous Administration of [^{14}C]Indigo (TBR[a])

	0.25 hr	0.75 hr	2.0 hr	6.0 hr	24.0 hr	72.0 hr	240.0 hr[d]
Skin (ears)	0.21 ± 0.05	0.27 ± 0.07	0.29 ± 0.01	0.38 ± 0.03	0.72 ± 0.21	0.86[b]	1.2 ± 0.6
(belly)	0.20 ± 0.03	0.23 ± 0.05	0.13 ± 0.12	0.30 ± 0.04	0.36 ± 0.04	0.56 ± 0.08	2.3 ± 1.4
(hindquarters)	0.12 ± 0.02	0.17 ± 0.04	0.19 ± 0.02	0.37 ± 0.14	0.30 ± 0.01[c]	0.53 ± 0.53	1.1 ± 0.2
(back of neck)	0.16 ± 0.04	0.18 ± 0.04	0.20 ± 0.02	0.36 ± 0.14	0.42 ± 0.18	0.52 ± 0.08	0.94 ± 0.38
Esophagus	0.65 ± 0.49	0.28 ± 0.02	0.32 ± 0.09	0.41 ± 0.08	0.97 ± 0.63	0.59 ± 0.08	
Stomach	0.77 ± 0.21	0.95 ± 0.61	1.2 ± 0.2	0.58 ± 0.19	0.32 ± 0.13	0.61 ± 0.43	
Liver	36 ± 13	39 ± 6	51 ± 13	74 ± 5	71 ± 10	66 ± 7	58 ± 8
Lungs	30 ± 14	24 ± 10	33 ± 10	55 ± 8	100 ± 27	67 ± 8	220 ± 20
Heart	1.1 ± 0.3	0.88 ± 0.12	0.81 ± 0.04	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	
Kidneys	3.1 ± 0.8	3.3 ± 0.61	2.6 ± 0.2	3.6 ± 0.4	5.6 ± 1.2	6.7 ± 0.9	17 ± 2
Adipose (kidney)	0.20 ± 0.07	0.23 ± 0.08	0.24 ± 0.02	0.21 ± 0.11	0.39 ± 0.18	0.26 ± 0.03	0.36 ± 0.10
(epididymis)	0.12 ± 0.02	0.15 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	0.27 ± 0.10	0.31 ± 0.10	0.18 ± 0.08
(mesenteric)	0.26 ± 0.06	0.34 ± 0.10	0.29 ± 0.05	0.27 ± 0.11	0.33 ± 0.07	0.28 ± 0.07	0.42 ± 0.11
Adrenals	1.8 ± 0.7	1.3 ± 0.5	1.8 ± 0.4	3.4 ± 0.4	4.2 ± 0.6	8.3 ± 7.7	8.2 ± 1.0
Small Intestines	1.4 ± 0.5	5.8 ± 2.6	6.2 ± 3.8	4.3 ± 1.8	2.5 ± 0.2	4.6 ± 2.3	1.4 ± 0.1
Large Intestines	0.28 ± 0.09	0.27 ± 0.07	0.26 ± 0.03	20 ± 16	13 ± 3	9.6 ± 4.2	2.9 ± 1.4
Cecum	0.16 ± 0.08	0.14 ± 0.01	0.43 ± 0.25	54 ± 14	18 ± 3	7.5 ± 1.1	
Seminal Vesicles	0.13 ± 0.04	0.14 ± 0.01	0.12 ± 0.0	0.13 ± 0.03	0.18 ± 0.03	0.30 ± 0.18	
Testes	0.16 ± 0.03	0.19 ± 0.04	0.22 ± 0.02	0.25 ± 0.02	0.32 ± 0.06	0.46 ± 0.03	
Prostate	0.21 ± 0.10	0.19 ± 0.03	0.17 ± 0.03	0.24 ± 0.14	0.22 ± 0.03	1.1 ± 0.7	
Muscle (neck)	0.26 ± 0.06	0.21 ± 0.01	0.17 ± 0.03	0.19 ± 0.03	0.24 ± 0.06	0.41 ± 0.18	0.48 ± 0.34
(hind leg)	0.23 ± 0.08	0.21 ± 0.03	0.18 ± 0.03	0.22 ± 0.06	0.18 ± 0.03	0.22 ± 0.14[c]	0.35 ± 0.17
(abdomen)	0.19 ± 0.05	0.20 ± 0.03	0.16 ± 0.02	0.23 ± 0.05	0.19 ± 0.04	0.26 ± 0.04	0.38 ± 0.19
Brain	0.50 ± 0.14	0.33 ± 0.07	0.32 ± 0.02	0.32 ± 0.08	0.26 ± 0.03	0.14 ± 0.06	
Eyes	0.12 ± 0.02	0.13 ± 0.01	0.12 ± 0.02	0.14 ± 0.03	0.17 ± 0.01	0.28 ± 0.09	
Spleen	27 ± 11	23 ± 6	31 ± 3	64 ± 7	100 ± 20	90 ± 25	180 ± 13
Plasma	1.2 ± 0.2	1.3 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	0.95 ± 0.16	0.80 ± 0.09	0.48 ± 0.06
Blood	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	9.5 ± 0.6	1.0 ± 0.0

[a]Values are the averages for 3 rats ± SD; Original report appendix has data from individual rats.

[b]Data for 1 rat only

[c]Data for 2 rats only

[d]Data for 4 rats.

Table 13

Amount of ^{14}C Contained in Selected Tissues after Intravenous Administration of 0.2 mg/kg of [^{14}C]Indigo (% Dose[a])

Time (hr)	0.25	0.75	2.0	6.0	24.0	72.0	240.0[b]
Skin	1.5 ± 0.2	1.8 ± 0.3	1.1 ± 0.2	0.96 ± 0.04	0.74 ± 0.15	0.38 ± 0.04	
Esophagus	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Stomach	0.29 ± 0.01	0.35 ± 0.24	0.32 ± 0.08	0.12 ± 0.08	0.03 ± 0.01	0.02 ± 0.01	
Liver	63 ± 5	57 ± 1	47 ± 4	43 ± 1	23 ± 1	11 ± 0.0	2.8 ± 0.1
Lungs	5.2 ± 1.2	3.6 ± 1.3	2.0 ± 0.5	3.7 ± 0.4	3.2 ± 1.0	1.2 ± 0.2	1.1 ± 0.1
Heart	0.19 ± 0.01	0.13 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.55 ± 0.15
Kidneys	1.04 ± 0.09	0.91 ± 0.14	0.45 ± 0.08	0.39 ± 0.03	0.32 ± 0.03	0.20 ± 0.02	0.15 ± 0.02
Adipose	1.0 ± 0.2	1.3 ± 0.1	0.83 ± 0.13	0.42 ± 0.09	0.30 ± 0.07	0.12 ± 0.03	0.04 ± 0.01
Adrenals	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cecum	0.10 ± 0.02	0.10 ± 0.02	0.19 ± 0.13	12 ± 3	2.9 ± 0.7	0.62 ± 0.03	
Seminal Vesicles	0.03 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Testes	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	
Prostate	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Muscle	6.1 ± 0.6	5.6 ± 0.5	2.6 ± 0.9	2.0 ± 0.4	0.96 ± 0.21	0.75 ± 0.23	0.28 ± 0.09
Brain	0.15 ± 0.03	0.09 ± 0.02	0.06 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	
Eyes	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Spleen	2.2 ± 0.6	1.8 ± 0.5	2.3 ± 1.5	1.7 ± 0.2	1.4 ± 0.1	0.77 ± 0.12	0.33 ± 0.08

[a]Values are the averages ± S.D. for 3 rats. Original report appendix has data from individual rats.
[b]Values are the averages ± S.D. for 4 rats.
[c]Adipose assumed to be 10% of body weight; muscle, 50% of body weight; and skin, 15% of body weight.

Table 14

Concentration of ^{14}C-Labelled Compounds in Tissues 96 hr after Oral Administration of [^{14}C]Indigo (ng-eq/g[a])

Dose (mg/kg)	2.97	31.1	305
Skin (Ears)	23 ± 4	120 ± 12	650 ± 100
(Belly)	24 ± 6	86 ± 11	590 ± 100
(Hindquarters)	15 ± 1	110 ± 9	600 ± 190
(Back of Neck)	19 ± 1	100 ± 6	620 ± 120
Liver	79 ± 27	530 ± 130	4000 ± 800
Lungs	19 ± 4	97 ± 12	670 ± 120
Kidneys	340 ± 61	2500 ± 100	14000 ± 2000
Adipose (Kidney)	38 ± 26	28 ± 10	190 ± 140
(Epididymis)	17 ± 5	24 ± 19	350 ± 90
(Mesenteric)	47 ± 25	49 ± 12	380 ± 110
Adrenals	47 ± 13	98 ± 13	720 ± 240
Muscle (Neck)	13 ± 1	32 ± 6	150 ± 130
(Hind Leg)	15 ± 3	18 ± 4	160 ± 70
(Abdomen)	14 ± 5	27 ± 1	210 ± 50
Spleen	21 ± 1	120 ± 20	1000 ± 260
Small Intestine	13 ± 3	59 ± 10	450 ± 30
Large Intestine	47 ± 11	450 ± 150	6700 ± 3600
Plasma	11 ± 5	63 ± 5	310 ± 60
Blood	20 ± 5	150 ± 20	1300 ± 60

[a]Values are the averages for 3 rats ± S.D. Original report appendix has data from individual rats.

Table 15

Tissue-Blood Ratios of ^{14}C-Labeled Compounds 96 hr after Oral Administration of [^{14}C]Indigo[a]

Dose (mg/kg)	2.97	31.1	305
Skin (Ears)	1.1 ± 0.1	0.77 ± 0.02	0.48 ± 0.06
(Belly)	1.2 ± 0.1	0.56 ± 0.01	0.43 ± 0.06
(Hindquarters)	0.78 ± 0.20	0.69 ± 0.08	0.44 ± 0.14
(Back of Neck)	0.98 ± 0.29	0.68 ± 0.07	0.45 ± 0.08
Liver	3.9 ± 0.7	3.4 ± 0.4	3.0 ± 0.6
Lungs	0.97 ± 0.24	0.62 ± 0.02	0.50 ± 0.08
Kidneys	17 ± 1	17 ± 2	10 ± 2
Adipose (Kidney)	2.0 ± 1.3	0.18 ± 0.06	0.14 ± 0.11
(Epididymis)	0.81 ± 0.03	0.16 ± 0.13	0.26 ± 0.07
(Mesenteric)	2.2 ± 1.0	0.43 ± 0.20	0.28 ± 0.08
Adrenals	2.4 ± 0.6	0.65 ± 0.05	0.53 ± 0.19
Muscle (Neck)	0.67 ± 0.17	0.21 ± 0.06	0.11 ± 0.10
(Hind Leg)	0.77 ± 0.29	0.12 ± 0.03	0.12 ± 0.05
(Abdomen)	0.73 ± 0.34	0.18 ± 0.03	0.16 ± 0.04
Spleen	1.0 ± 0.2	0.78 ± 0.03	0.75 ± 0.18
Small Intestine	0.64 ± 0.19	0.39 ± 0.05	0.33 ± 0.11
Large Intestine	2.7 ± 0.3	3.0 ± 1.2	2.5 ± 1.3
Plasma	0.50 ± 0.16	0.42 ± 0.08	0.23 ± 0.04
Blood	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

[a]Tissue blood ratios expressed as averages for 3 rats ± S.D. Original report appendix has data from individual rats.

Table 16

Amount of ^{14}C Contained in Selected Tissues 96 hr after Oral Administration of [^{14}C]Indigo (% Dose[a])

Dose (mg/kg)	2.97	31.1	305
Skin[b]	0.09 ± 0.02	0.05 ± 0.01	0.03 ± 0.01
Liver	0.09 ± 0.03	0.06 ± 0.02	0.04 ± 0.01
Lungs	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kidneys	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Adipose[b]	0.11 ± 0.05	0.01 ± 0.00	0.01 ± 0.00
Adrenals	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle[b]	0.21 ± 0.04	0.04 ± 0.00	0.03 ± 0.01
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[a]Values are the averages for 3 rats. Original report appendix has data from individual rats.

[b]Adipose assumed to be 10% of body weight; muscle, 50% of body weight; and skin, 15% of body weight.

Table 17

Concentration of ^{14}C in Tissues 192 hr After Topical Administration of [^{14}C]Indigo

	1.28 mg/kg as dry powder[a]			3.70 mg/kg in an ointment base[b]		
	ng-eq/g	TBR	% Dose per Tissue	ng-eq/g	TBR	% Dose per Tissue
Skin (ears)	1.79 ± 1.27	4.18 ± 3.22	0.018 ± 0.11	3.97 ± 2.29	1.57 ± 1.16	0.018 ± 0.004
(hindquarters)	1.36 ± 0.73	3.15 ± 1.95		4.90 ± 0.56	1.81 ± 0.15	
Liver	0.45 ± 0.08	0.95 ± 0.05	0.001 ± 0.000	1.13 ± 0.06	0.43 ± 0.11	0.001 ± 0.000
Lungs	0.14 ± 0.01	0.30 ± 0.04	0.000	0.87 ± 0.01	0.32 ± 0.05	0.000
Kidneys	1.09 ± 0.09	2.45 ± 0.45	0.000	2.97 ± 0.25	1.11 ± 0.09	0.000
Adipose (kidney)	0.82 ± 0.49	1.70 ± 0.90		0.86 ± 0.26	0.33 ± 0.15	
(epididymis)	0.46 ± 0.13	1.01 ± 0.39	0.004 ± 0.000[e]	0.35 ± 0.11	0.13 ± 0.02	0.002 ± 0.001[e]
(mesenteric)	0.32 ± 0.21	0.76 ± 0.54		0.67 ± 0.38	0.27 ± 0.20	
Adrenals	0.48 ± 0.31	1.10 ± 0.80	0.000	0.80 ± 0.26	0.31 ± 0.15	0.000
Muscle (neck)	0.25 ± 0.01	0.55 ± 0.09		1.33 ± 0.49	0.50 ± 0.16	
(hind leg)	0.12 ± 0.06	0.25 ± 0.10	0.006 ± 0.002[e]	0.45 ± 0.35	0.18 ± 0.17	0.009 ± 0.003[e]
(abdomen)	0.07 ± 0.03	0.14 ± 0.05		0.30 ± 0.15	0.11 ± 0.06	
Spleen	0.22 ± 0.01	0.47 ± 0.08	0.000	0.64 ± 0.45	0.38 ± 0.06	0.000
Clean Small Intestine	0.47 ± 0.25	1.11 ± 0.69		0.55 ± 0.23	0.20 ± 0.06	
Clean Large Intestine	0.48 ± 0.31	1.10 ± 0.80		2.29 ± 1.04	0.92 ± 0.59	
Small Intestine	0.09 ± 0.01	0.18 ± 0.04		2.58 ± 1.64	0.91 ± 0.49	
Large Intestine	0.94 ± 0.42	2.14 ± 1.16		9.07 ± 6.27	3.18 ± 1.85	
Muscle[d]	--- ---	--- ---		0.57 ± 0.13	0.22 ± 0.07	
Subcutaneous Fat[d]	1.62 ± 0.95	3.8 ± 2.5		2.01 ± 0.37	0.75 ± 0.16	
Brown Fat[d]	2.64 ± 0.26	5.7 ± 0.1		3.73 ± 2.16	1.49 ± 1.13	
Dermis[d]	5.1 ± 5.1	12.5 ± 12.5		740 ± 500	270 ± 170	
Patch			66.6 ± 1.1			45.5 ± 12.5
Epidermis under Patch			8.04 ± 2.26			46 ± 13.4
Plasma	0.00	0.00		0.09 ± 0.09	0.03 ± 0.03	
Blood	0.47 ± 0.06	1.00 ± 0.00		2.77 ± 0.49	1.00 ± 0.00	

[a] Average of 2 Rats ± Range. Original report appendix has data from individual rats.

[b] Average of 3 Rats ± S.D. Original report appendix has data from individual rats.

[c] Total dose applied.

[d] From under patch.

[e] Adipose assumed to be 10% of body weight; muscle, 50% of body weight; and skin, 15% of body weight.

Table 18

Concentration of Parent Compound in Liver at Various Times Following an Intravenous Dose of 0.22 mg/kg or an Oral Dose of 31 mg/kg of [^{14}C]Indigo

Time (hr)	Route	Rat No.	Indigo (µg/g tissue)	% of Total ^{14}C in Tissue Accounted for by Indigo
0.25	IV	I39	1.05	23.0
		I41A	0.62	13.2
		I46	1.19	31.9
		Average ± S.D.	0.95 ± 0.3	22.7 ± 9.4
6.0	IV	I33	0.58	18.5
		I34A	1.37	42.8
		I35A	1.16	38.1
		Average ± S.D.	1.04 ± 0.41	33.1 ± 12.9
72	IV	I2	0.20	31.6
		I3	0.25	42.3
		I28	0.21	29.8
		Average ± S.D.	0.22 ± 0.03	34.6 ± 6.8
96	Oral	I16A	<0.0005	0.07
		I17	<0.0005	0.01
		I18	<0.0005	0.01
		Average ± S.D.		0.03 ± 0.03