♦ TOXICOLOGY

Toxicology studies (GLP) with icodextrin solutions were conducted at:

1. Single Dose (IP and IV routes)

1.1 MOUSE

SINGLE DOSE TOXICITY					
Ref. to document: Section 8, Attachment 6 Report date: Oct. 1990 Project Number: — 243247. Rept. No. 6466 Study period: 1990 .					
Species/Strain: MOUSE /CD1					
Administration route: INTRAPERITONEAL			Number of animals: 10		
20% ICODEXTRIN ADMINISTERED AT 10 ML.KG^{-1}					
Treatment of controls: NO CONTROL GROUP			Observation period: (Appl. day - Day 1) 14 DAYS		
Study group	(1) Contr.		(2)		
Dosage <MG/KG>	0		2000		
Sex (m/f)	m	f	m	f	
Animals per dosage	0	0	5	5	
D E A T H					
			0	0	

Summary of salient findings

NO DEATHS FOLLOWING I.P. INJECTION OF ICODEXTRIN AT 2000 MG/KG, OR DURING OBSERVATION PERIOD.

NO CLINICAL SIGNS OR NECROPSY FINDINGS.

BODY WEIGHT GAIN WAS LOWER THAN EXPECTED

SINGLE DOSE TOXICITY					
Ref. to document: Section 8, Attachment 7 Report date: Oct. 1990 Project Number — 243247 Rept. No.: 6464 Study period: 1990					
Species/Strain: MOUSE /CD1					
Administration route: I.V.			Number of animals: 10		
20% ICODEXTRIN ADMINISTERED AT 5 ML.KG^{-1}					
Treatment of controls: NO CONTROL GROUP			Observation period: (Appl. day - Day 1) 14 DAYS		
Study group	(1) Contr.		(2)		
Dosage < MG/KG >	0		1000		
Sex (m/f)	m	f	m	f	
Animals per dosage	0	0	5	5	
D E A T H					
			0	0	

Summary of salient findings

NO DEATHS FOLLOWING ADMINISTRATION OF 20% ICODEXTRIN, OR DURING OBSERVATION PERIOD.

NO CLINICAL SIGNS OR NECROPSY FINDINGS

IND ___

Toxicology (Single Dose Cont'd.)

1.2 RAT

SINGLE DOSE TOXICITY					
Ref. to document: Section 8, Attachment 8 Report date: Oct. 1990 Project Number ___ 243247 Rept. No. 6465 Study period 1990.					
Species/Strain: RAT/SPRAGUE - DAWLEY					
Administration route: INTRAPERITONEAL			Number of animals: 10		
20% ICODEXTRIN ADMINISTERED AT 10ML.KG^{-1}					
Treatment of controls: NO CONTROL GROUP			Observation period: (Appl. day - Day 1) 14 DAYS		
Study group	(1) Contr.		(2)		
Dosage <MG/KG>	0		2000		
Sex (m/f)	m	f	m	f	
Animals per dosage	0	0	5	5	
DEATH			0	0	
Summary of salient findings NO DEATHS FOLLOWING I.P. INJECTION OF ICODEXTRIN AT 2000 MG/KG, OR DURING OBSERVATION PERIOD. NO CLINICAL SIGNS OR NECROPSY FINDINGS					

SINGLE DOSE TOXICITY					
Ref. to document: Section 8, Attachment 8 Report date: Oct. 1990 Project Number:243247 Rept. No. 6463 Study period 1990.					
Species/Strain: RAT /SPRAGUE - DAWLEY					
Administration route: I.V.			Number of animals: 10		
20% ICODEXTRIN ADMINISTERED AT 5 ML.KG^{-1}					
Treatment of controls: NO CONTROL GROUP			Observation period: (Appl. day - Day 1) 14 DAYS		
Study group	(1) Contr.		(2)		
Dosage <MG/KG>	0		1000		
Sex (m/f)	m	f	m	f	
Animals per dosage	0	0	5	5	
DEATH			0	0	
Summary of salient findings NO DEATHS FOLLOWING ADMINISTRATION OF 20% ICODEXTRIN, OR DURING OBSERVATION PERIOD. NO CLINICAL SIGNS OR NECROPSY FINDINGS					

Single dose toxicity studies: vol. 1.7 p.366-417

This includes 4 studies from [redacted] numbers 6464, 6466, 6463, and 6465. Although these toxicology studies were negative, dosages used were less than those proposed for therapeutic usage. The dosage used in all the studies was 10 ml/kg of a 20% icodextrin solution equivalent to a dosage of 2g/kg. The proposed human dose is approximately 2000 to 2500 ml/70kg adult of 7.5% icodextrin, a dosage of 2.14-2.7 g/kg.

Study #	Dosage	Route of Administration	Results
6464	5 ml/kg	Intravenous	No toxicity seen
6466	10 ml/kg	Intraperitoneal	No toxicity seen
6463	5 ml/kg	Intravenous	No toxicity seen
6465	10 ml/kg	Intraperitoneal	No toxicity seen

Therefore, these studies are not useful for determining icodextrin toxicity.

Study # 9-292: The Single Dose Toxicity Test of Icodextrin by Intraperitoneal Administration in Rats vol 1.7 p.418-449

44 Sprague Dawley rats at 5 weeks of age were obtained for this study, 22 males weighing between 132.9 to 149.1 g and 22 females between 119.8 to 132.3 g. 10 animals (5 of each sex) were randomly allocated to receive either electrolyte solution, 15 g/kg, 25 g/kg, or 50 g/kg of icodextrin solution. All groups received an intraperitoneal injection volume of 200 ml/kg except for the 25 g/kg group, which received 100 ml/kg. Animals were monitored for 15 days after injection, and survivors were sacrificed on day 16. An autopsy involving a macroscopic examination of the various major organ systems was performed.

2 males died in the electrolyte group, 2 males died in the 15 g/kg icodextrin group, 1 male died in the 50 g/kg icodextrin group, and 2 females died in the 15 g/kg icodextrin group. 5 of 20 males died and 1 of 20 females in this study. Abdominal distension was the primary adverse reaction with some experiencing piloerection and tachypnea, generally resolving within 5 days. Weight gain was similar among all the groups.

Unfortunately the study did not use a true control, but used the pH 5.5 electrolyte solution. Although in other studies a large volume was used, for example the blood volume study also used 200 ml/kg, and used a saline control. The blood volume studied demonstrated that the electrolyte solution alone perturbed the physiology of the test animals, therefore it is inappropriate to use as a control.

Comment on the gender differences in mortality, may be due to differences in metabolism.

Study 9-314 vol 1.8, p. 1-25

The Single Dose Toxicity Test of Icodextrin in Rats by Intraperitoneal Administration at a Large Volume.

This study was a follow up to study # 9-292 and used the same methodology except only two groups, electrolyte solution and 7.5% icodextrin solution. One male animal in the electrolyte solution group died. Abdominal distension was the primary adverse event, with some experiencing piloerection and tachypnea, as in study #9-292.

Although the study was to illuminate the deaths in the first study (9-292), it was uninstructive.

Multiple Dose Studies:

A. Rats The first section is reviews done by Estella Barry as part of a review for IND — followed by additional observations.

APPEARS THIS WAY
ON ORIGINAL

IND —— 12

2. Repeat Dose Studies

Listed below are the repeat dose studies conducted in rat and dog with various concentrations of icodextrin solutions.

Summary of Subchronic Toxicity Studies

	Strain	Initial Group	Mode Admin.	Dose g/kg/day	Duration	Interim Sacrifice	Report No.	Attach. No.
Rat	S-D	5M	IP	0,8.4,12	7	-	7390	10
	S-D	5M	IP	12	3,28	-	7400	11
	S-D	16M + 16F	IP	0,3,8 4,12	28	-	7423	12
Dog	Beagle	1M + 1F	IP	0,9.3,11, 37.6,44, 75.2,88	7	-	5062	13
	Beagle	4M + 2F	IP	varied	8 to 29	-	7652	14
	Beagle	3M + 3F	IP	0,3,8.4,12	28	-	7523	15

2.1 RAT 7-day IP Repeat Dose Pilot Study

This pilot 7-day rat study was to determine feasibility of twice daily administrations of 14% and 20% icodextrin solutions.

Report date: Dec 1991 Project No: —J 641907. Report No: 7390 Study period (years): 1990.								
Species/Strain: RAT/SPRAGUE - DAWLEY								
Number of animals: 15			Duration of treatment: 7 DAYS					
Observation period after the end of dosing: NONE								
Administration route INTRAPERITONEAL								
Treatment of controls: ELECTROLYTE SOLUTION ONLY			Age: NOT STATED at study Body weight: 222-231G initiation Treatment days per week:					
Study group	(1) CONTROL.		(2) ICODEXTRIN		(3) ICODEXTRIN			
Dosage <G/KG BID. >	C - 0		LD - 4.2		HD - 6.0			
Sex (m/f)	m	f	m	f	m	f	m	f
Number of test animals	5	-	5	-	5	-		
Number of animals died or sacrificed in extremis	0		0		0			
Clinical observations: yes			Clin. chemistry: yes					
Food consumption: yes			Urinalysis: no					
Water consumption: yes			Organ weights: no					
Body weight: yes			Necropsy: yes					
Hematology: yes			Histology: no					
Additional examinations: BLOOD SAMPLES ON DAY 8								
Additional informations: TEST MATERIAL ADMINISTERED USING DOSE VOLUME OF 30 ML/KG, TWICE DAILY. GROUP 2 RECEIVED 14% ICODEXTRIN, GROUP 3 RECEIVED 20% ICODEXTRIN.								

IND —— 13

Rat, 7-day pilot (Cont'd.)

Results:

Twice a day peritoneal injection with 30 ml/kg icodextrin solutions caused no deaths. Compared to control (treated with electrolyte solution alone), rats treated with the 14% and 20% icodextrin solutions showed reduced (↓) food consumption (at LD ↓19% and HD ↓22%), and body weight gain (↓15% and ↓27%, respectively.) No treatment related abnormalities were reported. In the HD animals, hematology showed a reduction in eosinophil counts ($p<0.01$) vs control. Clinical chemistry in drug treated groups revealed a reduction albumin, blood urea nitrogen levels ($p<0.001$, attributed by drug sponsor to reduction in food intake) and an increase in blood chloride levels. Necropsy revealed red areas around the caecum of one LD rat.

Drug sponsor concluded that Sprague-Dawley rats tolerated twice daily peritoneal injections of icodextrin (14% and 20%) at dosage volume of 30 ml/kg.

Drug sponsor concluded that in this study the MTD of icodextrin was 30 ml/kg ip of a 20% solution over 7 days.

APPEARS THIS WAY
ON ORIGINAL

IND —— 14

Toxicology (Cont'd.)

2.3 RAT 28-day IP Repeat Dose Pilot Study.

Because the solutions of icodextrin showed increased in viscosity with increasing concentrations, the drug sponsor performed a pilot study in a small number of rats to explore the maximum tolerated dose (MTD). Thus, the 20% icodextrin sol.* was given in a 30 ml/kg volume BID (to Group 1 for 3 days and to Group 2 for 28 days), and potential for recovery from the administered dose was determined.

This HD dose (20%) of icodextrin is estimated to be similar to that intended for clinical use (i.e., 2000 ml of a 7.5%icodextrin sol. for a 70 kg patient as ~ 29 ml/kg). It appears that the concentrations of the solutions are limited because of the increasing viscosity.

REPEATED DOSE TOXICITY Subchronic toxicity (up to 3 months)							
Ref. to document: Section 8, Attachment 11 Report date: Dec 1991 Project No: — 641886 Report No: 7400 Study period (years): 1990.							
Species/Strain: RAT/SPRAGUE - DAWLEY							
Number of animals: 10			Duration of treatment: 3-28 DAYS				
Observation period after the end of dosing: NONE							
Administration route INTRAPERITONEAL							
Treatment of controls: NO CONTROL GROUP			Age: NOT STATED at study initiation Body weight: 190-212G Treatment days per week: 3(GP1) 7(GP2)				
Study group			(1)		(2)		
Dosage <G/KG B.D>	0		6		6		
Sex (m/f)	m	f	m	f	m	f	
Number of test animals			5		5		
Number of animals died or sacrificed in extremis			0		1		
Clinical observations: yes			Clin. chemistry: no				
Food consumption: no			Urinalysis: no				
Water consumption: yes			Organ weights: no				
Body weight: yes			Necropsy: yes				
Hematology: no			Histology: no				
Additional examinations: PERITONEAL FLUID RECOVERY							
Additional informations: GROUP 1 WAS SACRIFICED AT 3 DAYS GROUP 2 AT 28 DAYS. TEST MATERIAL WAS ADMINISTERED USING DOSE VOLUME OF 30 ML/KG, TWICE DAILY. AT NECROPSY SPECIAL ATTENTION WAS GIVEN TO APPEARANCE OF ABDOMINAL ORGANS. THIS WAS A PILOT FEASIBILITY STUDY.							

Results

The most remarkable effect reported was death on day 13 in 1 M from Group 2. (Death was attributed by sponsor to the technical procedure not the drug).

* Batch # 90C22BF

IND [redacted] 15

Peritoneal fluid was collected from some rats from Group 2 on isolated occasions. Three rats were sacrificed after 3 days of dosing showed fluid in the abdominal cavity. Rats sacrificed after 28 days of treatment showed subcutaneous hemorrhage of the abdominal wall and the abdominal fat showed a gelatinous appearance.

Drug sponsor concluded that is was feasible to administer 20% icodextrin solution by ip injection to rats for up to 28 days in a volume of 30 ml/kg.

2.2 **RAT** 28-day IP Repeat Dose Formal (with 14-day recovery period) Study.

The purpose of this definitive study was to investigate the toxicity of BID (7 hrs apart) ip dosing with icodextrin solutions (14%* and 20%**) to rats (19/sex/group) for 28 days compared to vehicle control (electrolyte solution) and to a 5% glucose sol., which is commonly used for PDS. Test materials were administered over ~10-60 seconds.

The dose levels of icodextrin were selected by drug sponsor to maximize peritoneal exposure and they are equivalent to up to 3 X the intended clinical use concentration.

Serum, urine and dialysis fluid were obtained in this study were analyzed for icodextrin and metabolites. An **assay was developed** to separate and analyses the components of glucose polymer in plasma, dialysis and urine samples. The assay is described (precision and accuracy also reported) in the submission.

At the end of the dosing period, 6 rats/sex/icodextrin and controls groups were observed for an additional 14 days (days 28 to 42 of study) to investigate the reversibility of any effect observed.

Observations for the definitive and 14-day recovery study are summarized by sponsor in the table below, edited by reviewer.

REPEATED DOSE TOXICITY Subchronic toxicity (up to 3 months)						
Ref. to document: Section 8, Attachment 12 Report date: Project Number: [redacted] Rept. No. 7423 Study period 1990.						
Species/Strain: RAT/SPRAGUE - DAWLEY						
Number of animals: 126			Duration of treatment: 28 DAYS			
Observation period after the end of dosing: 14 DAYS; GROUPS 1,3,4 RECOVERY						
Administration route INTRAPERITONEAL						
Treatment of controls: I.P. INJECTION OF ELECTROLYTE SOLUTION ONLY			Age: at study initiation Body weight: 196-228 G Treatment days per week: 7			
Study group	(1) RECOVERY		(3) RECOVERY		(4) RECOVERY	
Dosage < G/KG. BD. >	0		ICODEXTRIN 4.2		ICODEXTRIN 6.0	
Sex (m/f)	m	f	m	f	m	f
Number of test animals	3	3	3	3	3	3
Number of animals died or sacrificed in extremis	0	1	0	0	1	0
Clinical observations:	yes		Clin. chemistry:	yes		
Food consumption:	yes		Urinalysis:	no		
Water consumption:	yes		Organ weights:	yes		
Body weight:	yes		Necropsy:	yes		
Hematology:	yes		Histology	yes		
Additional examinations NONE						

* Batch # 90C21BF.
** Batch # 90C22BF

IND —— 16

Results:

Some rats showed red staining around snout, yellow staining around the genital areas and pale extremities. Four rats showing these signs during the study were sacrificed in extremis, 1F (control) on day 13, 1 LD F on day 5; 1 HD M on day 14, and 1 M was found dead on day 14 of study.

Signs reported in most rats included distended abdomen (attributable to ip solution) and slight hair loss. Severe diarrhea was reported in 2 HD M on icodextrin. Fluid was recovered occasionally from icodextrin and glucose treated rats.

Compared to control, except for the 5% glucose treated group who gained weight, icodextrin treated rats M tended to show a decrease in body weight gain throughout the study; the decrease was attributed to the decrease in food consumption in icodextrin treated rats. The icodextrin rats showed that their body weight continued to increase during the 14-day recovery period.

Hematology changes (slight ↓ MCH and MCV values in icodextrin treated M) and slight increases in WBC in F were not considered by drug sponsor to be of toxicologic significance. Clinical chemistry showed lower levels of BUN in icodextrin-treated rats; protein and albumin were reduced in HD M compared to controls. However, all changes were reported to be within expected laboratory reference ranges by drug sponsor.

Remarkable ultrastructural histologic observations consisted in increase incidence in the level of vacuolation in the cytoplasm of hepatocytes of M treated with LD and HD icodextrin solutions.

Urinalysis revealed an increase in volume; decreases in urinary K^+ and Na^+ were reported for icodextrin treated rats.

At the end of 28 days of treatment, animals were killed and the absolute weight of 14 organs were recorded. Except for a significant decreased ($p<0.05$) in thyroid absolute weight in the 5% glucose treated M rats, no other organs showed a remarkable change when compared to controls.

At necropsy, a number of rats showed edema and inflammatory areas around the site of injection and increase size in the large intestine which was considered as an inflammatory reaction due to the method of dosing.

From the observed findings, drug sponsor concluded that there was no irritancy/toxicity of any of the abdominal organs/tissues associated with peritoneal administration of icodextrin solutions to rats for 28 days.

IND —— 17

♦ Recovery Period Study.

In the 14-day recovery study rats, compared to control, LD M showed a reduction in kidney weights ($p<0.05$); MD and HD F showed an increase in brain weights ($p<0.05$ and $p<0.01$ respectively). No histologic changes related to treatment were reported.

Study 7390

This study was to determine if it was feasible to give icodextrin in twice daily intraperitoneal injections of 30 ml/kg to Sprague-Dawley rats.

The study demonstrated that rats tolerated the icodextrin treatment. The rats treated for 7 days with 14 and 20% icodextrin did have a reduction in food consumption and body weight. All animals grew, however rats treated with 14% icodextrin were 85% of the controls, while the rats treated with 20% icodextrin were 73% of the controls. Food consumption declined similarly, 81 and 78% respectively for 14 and 20% icodextrin. With regards to clinical chemistry parameters, icodextrin treated rats showed an increase in chloride levels and a decrease in serum albumin compared to electrolyte solution treated controls.

Study 7400

This study was more designed to examine the feasibility of recovering fluid from the peritoneal cavity following injection with a volume of electrolyte or icodextrin solution. The study was also designed to test whether the rats (10 animals) could successfully handle twice a day injections of 20% icodextrin solution at 30 ml/kg, approximately the human volume and 2 2/3 times the human dosage. Basically, the study results coincided with those of Study 7390, with the exception that peritoneal fluid recovery was attempted. Only intermittent recovery of small volumes was possible with a lot of inconsistency in the results, and many animals yielding no recovered perioneal fluid. Therefore, it was found that the animals would tolerate the twice a day intraperitoneal injection of icodextrin, however recovery of peritoneal fluid was not practical.

Study 7423: Dextrin Polymer 28 day peritoneal toxicity study in rats vol 1.8, p. 159-369

This study was the primary rat toxicity study. Animals from this study were also utilized for the rat pharmacokinetics study (vol. 1.11, p. 214-243). As with many other of the submitted studies, using the electrolyte solution as a control decreases the opportunity to examine the true effects. Approximately 7.5 ml of pH 5.0 solution is being injected into animals with a total blood volume of 15 ml. Another concern is the misrepresentation of 5% Glucose as a typical peritoneal dialysis concentration. Generally, 1.36, 2.27, and 3.86% glucose are the light, normal, and heavy dialysis solutions, with the

heavy pulling more water from the patient. 5% glucose should be regarded as double the normal glucose concentration, and more similar to the 14% icodextrin solution, rather than being treated as a comparison to a normal dialysis solution.

Of concern to this reviewer is the potential emergence of gender specific issues in the animal studies. For example, in the Appendix 4 (p.255) clinical signs tables, of the 63 male rats, 28 of 63 animals had adverse clinical signs, while with the females, 6 of 63 animals had adverse signs.

APPENDIX 4

Dextrin Polymer
28 Day Peritoneal Toxicity Study in Rats
Clinical Signs: Males

Group/Treatment (30 ml.kg^{-1} b.d)	Animal	Day of Study	Observation
1 (Electrolyte Solution)	2	22-28	Scab on right shoulder
	4	9	Slight hair loss
	8	21	Small lump lower abdomen
	11	3-12	Scab on right shoulder
		13-18	Scabbing both shoulders
		18-28	Red/moist scabbing both shoulders
	18	22-28	Scab on right shoulder
2 (5% Glucose)	42	3	Distended abdomen
	44	3	Distended abdomen
3 (14% Dextrin Polymer)	53	22-28	Scab on left shoulder
	57	3	Distended abdomen
	58	3	Distended abdomen
	59	3	Distended abdomen
	61	15-16	Slight scabbing both shoulders. Right shoulder red/moist
		22-28	Scabbing both shoulders. Left shoulder red/moist
	62	22-28	Scab on left shoulder
	63	22-28	Scab on left shoulder
	66	3	Distended abdomen
		22-28	Scab on left shoulder
	68	3	Distended abdomen
		23-28	Scab on left shoulder

Clinical Signs: Males (cont'd)

Group/Treatment (30 ml.kg^{-1} b.d)	Animal	Day of Study	Observation
	89	22-28	Slight hair loss and discolouration of forelimbs
	90	3	Distended abdomen
		25	Severe diarrhoea
	92	3	Distended abdomen
	93	3	Distended abdomen
	94	3	Severe diarrhoea
	98	3	Severe diarrhoea
		27	Scabbing on left ear
		29	Dry red encrustation on left ear
4	99	3	Distended abdomen
(20% Dextrin	100	3	Distended abdomen
Polymer)	101	3	Distended abdomen
		13	Very subdued, red staining around snout, piloerection, extremities pale
		14	Animal killed in extremis
	104	3	Distended abdomen
		13	Very subdued, red staining around snout, piloerection, extremities pale
		14	Animal found dead in cage
	105	3	Distended abdomen
		25	Diarrhoea
	107	21	Severe diarrhoea

Clinical Signs: Females

Group/Treatment [30 ml.kg^{-1} b.d]	Animal	Day of Study	Observation
1 (Electrolyte Solution)	30	11	Dark red faeces
		13	Very subdued, red staining around snout, yellow staining around genitals, extremities pale
			Animal killed in extremis
2 (5% Glucose)	-	-	No abnormalities detected
3 (14% Dextrin Polymer)	73	23-28	Scab on right shoulder
	74	29	Small encrustation on right scapular area
	78	4	Very subdued, red staining around snout and fore-paws, yellow staining around genital area
		5	Animal killed in extremis
	82	26	Slight hair loss
	87	3	Distended abdomen
4 (20% Dextrin Polymer)	-	-	No abnormalities detected

Necropsy results showed glycogen deposits in the 14% and 20% icodextrin groups in the liver. In the 20% group, 4/13 females had positive kidney results, with basophilic staining probably indicative of nephropathy. In the males, 5/13 experienced testicular inflammation. Interestingly, one of the female rats in the 14% developed a mass and pale patches on the left kidney. The 5% glucose group had only minor findings from the necropsies, while the electrolyte solution treated group had 6/13 females with kidney findings, 9/13 males with testicular effects (including inflammation and atrophy), and 5/13 males with kidney findings. Spontaneous kidney nephropathy is a common occurrence in male sprague-dawley rats, however, it is not common in female sprague-dawley rats, thus indicating a different mechanism and cause for concern. Many of the necropsy results resolved during the 14 day recovery period.

Most of the damage is apparent with the kidney-related results. The change in urinary parameters, decrease sodium excretion, while in a positive water balance situation (see rat pk study), and the histological findings all point to damage to the kidneys, especially from the electrolyte solution alone. This solution, being added in a large volume, equal to approximately half the total blood volume, may cause acidosis since it is a lactate buffered solution at pH 5.0 –6.0. Studies of bicarbonate levels and respiration rates could have provided insight.

In addition, alkaline phosphatase and LDH levels were elevated in all the animals, with phosphate levels below the normal clinical range levels in female rats. In the recovery period, alkaline phosphatase levels

continued to increase in males, and phosphate levels continued their decline in females. LDH levels moderated in both sexes, and alkaline phosphatase levels remained high in females.

There was a corresponding decrease in sodium and potassium excretion. In females, creatinine clearances were 49 to 59 % of the male values and normal values, adding to the gender differences seen in the PK study.

Relating more strictly to the safety of icodextrin solution, increased glycogen depostion in the liver and effects on kidney are the largest concerns.

Interestingly, and corresponding with the ECG results seen in the General Pharmacology study, in the rats some cardiomyopathy was observed. Male rats in the electrolyte group were the most impacted with 5/13 effected, while on 1/13 females were effected. None of the rats in the 5% glucose or 14% icodextrin group showed signs of cardiomyopathy, but in the 20% icodextrin group 2/13 males and 3/13 females displayed an incidence of cardiomyopathy. Up to an enlargment of 20% occurred in male hearts, but was not seen in the females and recovered to normal size after removal from treament.

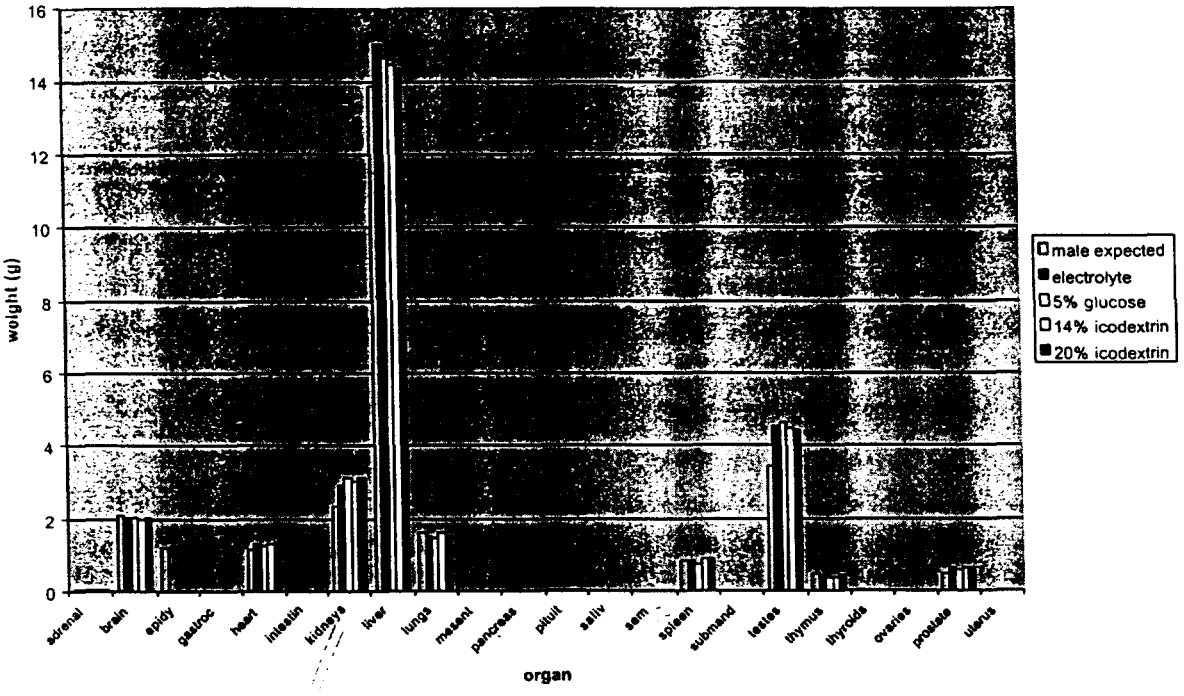
Table _. Organ weight changes in rats during the icodextrin study.

Males		Females	
Increased	Reduced	Increased	Reduced
Heart	Pituitary	Kidneys	Adrenal
Kidneys	Thymus	thyroids	Brain
Testes			Lungs
Thyroids			Pituitary
Prostate			Spleen (not 20% group)
			Thymus
			ovaries

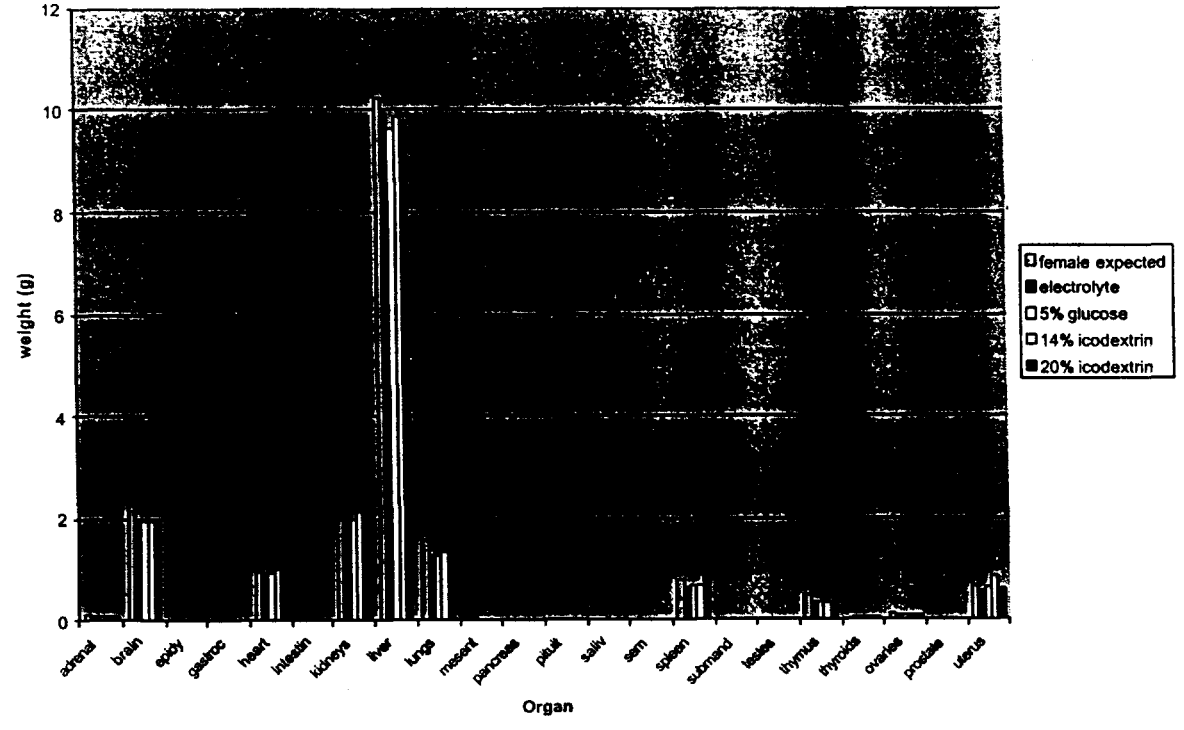
Table _. Organ weight changes in rats after recovery period.

Males		Females	
Increased	Reduced	Increased	Reduced
Kidneys	Lungs	thyroids	Lungs
Testes	Pituitary		Pituitary
Thyroids	Spleen		Spleen
prostate	thymus		Thymus
			ovaries

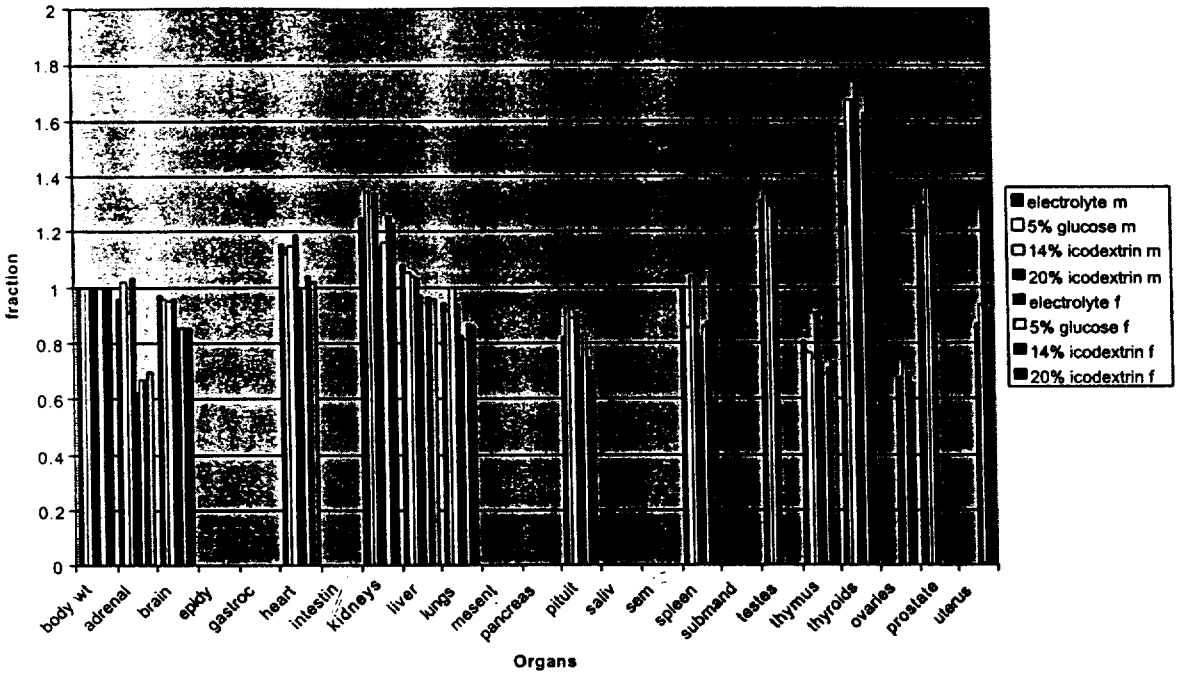
Male rat organ weights



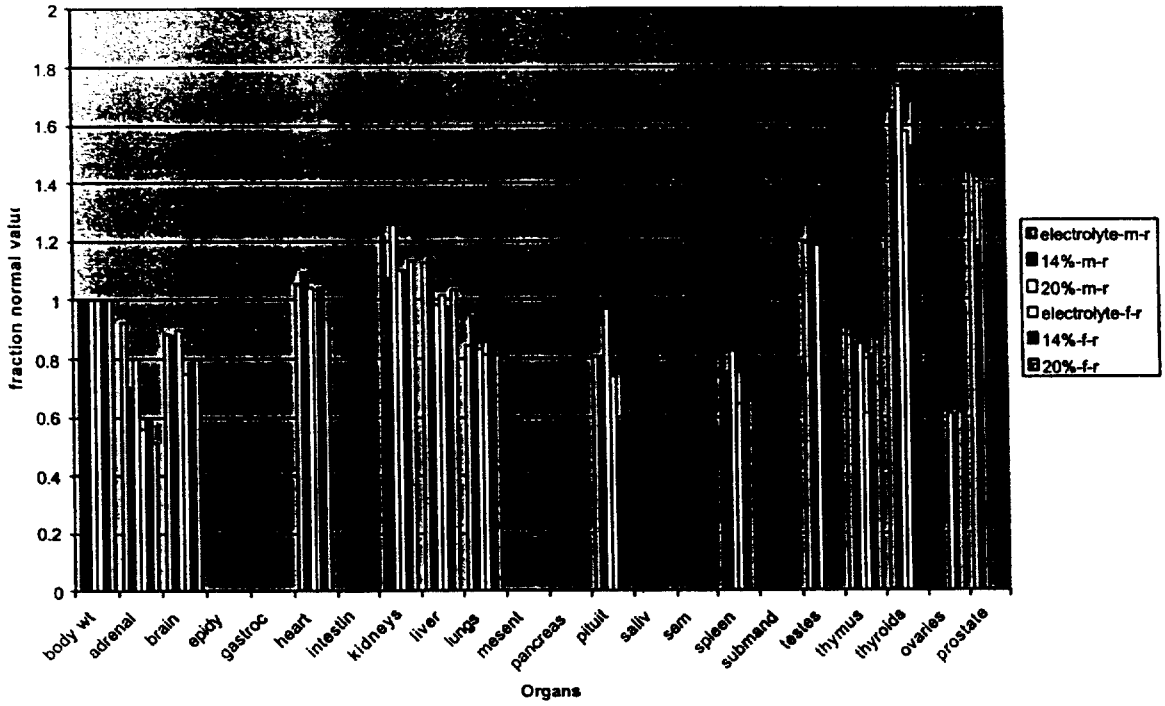
Female rat organ weights



male & female organ weights relative to normal values



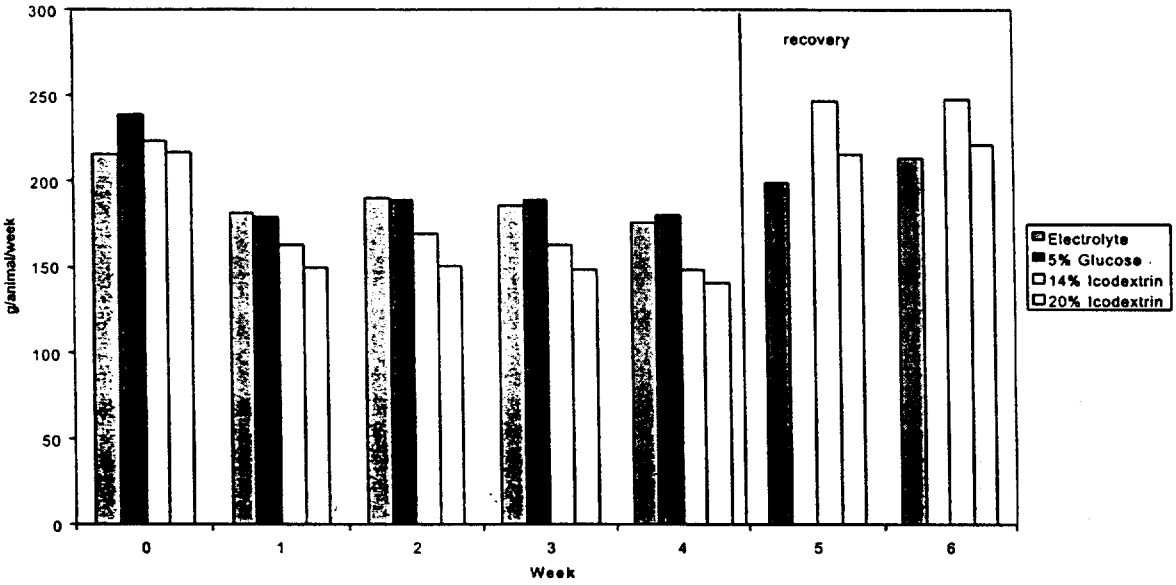
organ weights, male & female rats - recovery period



Other concerns: study time was too short, but by prior agreement.

Patients start dialysis with some residual kidney function, will icodextrin in PD-2 damage that function, end it quicker?

Food Consumption-males



Food Consumption-females

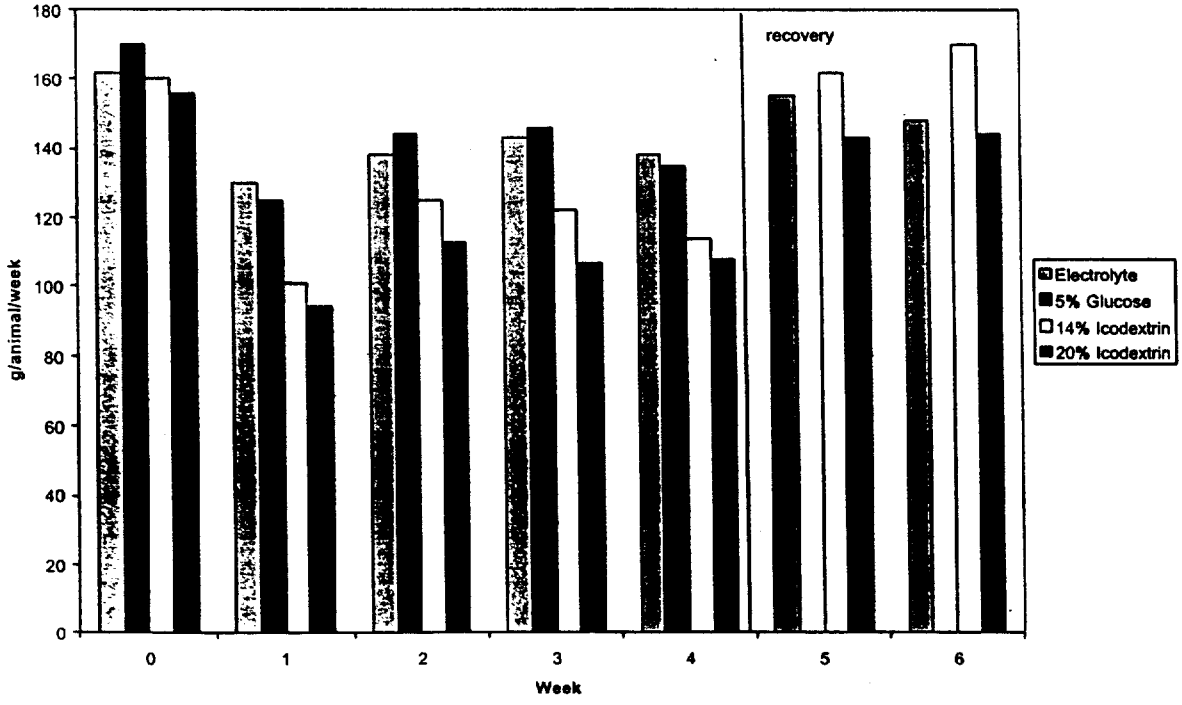


FIGURE 1

Dextrin Polymer
28 Day Peritoneal Toxicity Study in Rats
Group Mean Body Weight (g) : Males

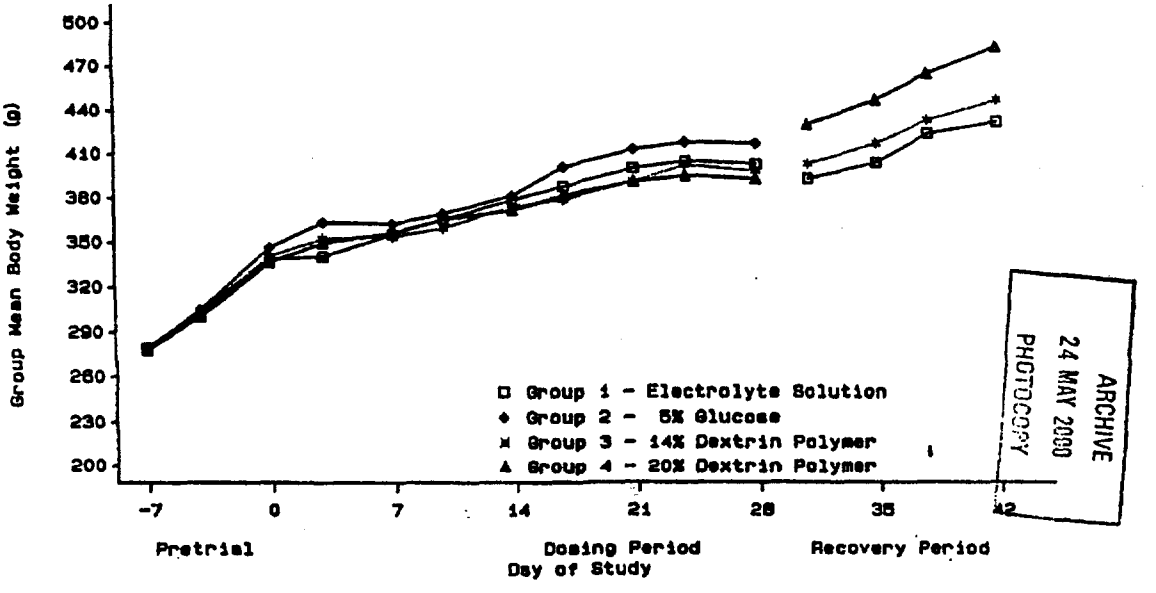
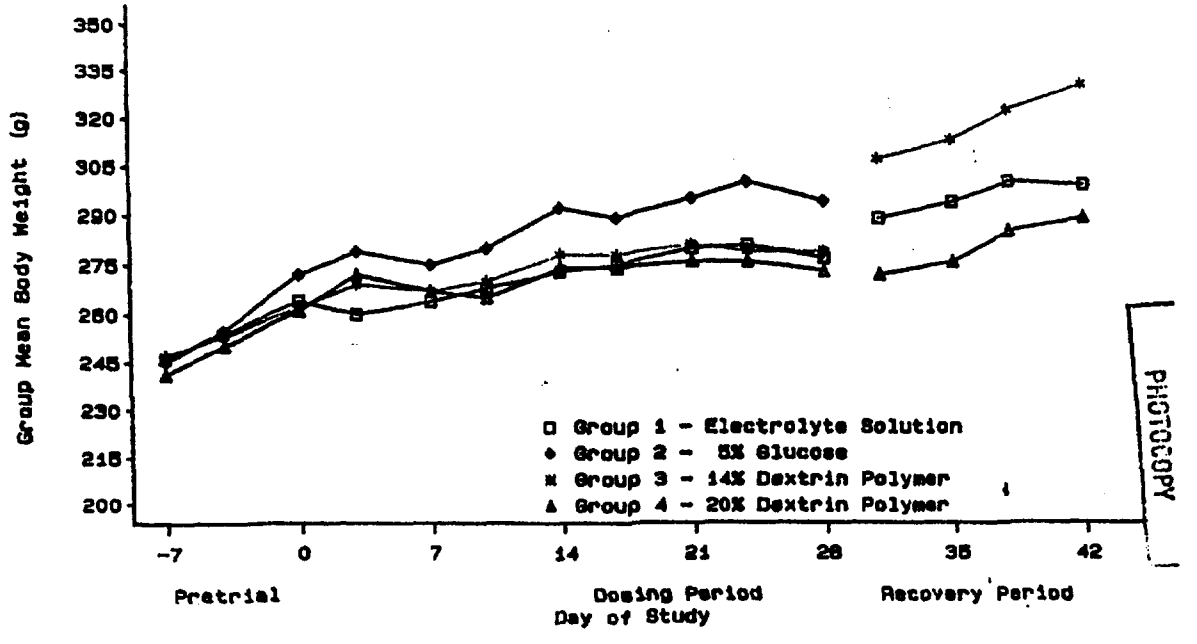


FIGURE 2

Dextrin Polymer
28 Day Peritoneal Toxicity Study in Rats
Group Mean Body Weight (g) : Females



In addition to the positive water balances, effects of treament on food consumption and body weight were of interest. All groups experienced a decrease in food consumption with the start of treatment. However, growth did continue, with males gaining approximately 60 g over the 4 week study period and females 15 g. The 5% glucose groups had the most weight gain over the 4 week period, and the 14 and 20 % icodextrin groups gained weight at the same rate as the electrolyte solution receiving group, despite a 30% drop in food consumption in the 20% icodextrin group.

Rats were also isosthenuric in the electrolyte and 5% glucose treatments, and marginally within the normal range for 14 and 20 % icodextrin. This is a further indication of kidney damage in that the kidney is no longer able to concentrate urine.

These results raise concern about the use of the electrolyte solution as a control. As was seen in the blood volume and electrolytes study, the electrolyte solution perturbed basic blood chemistry and volume very differently from a normal saline solution. And, in the EKG study in the General Pharmacology study, the electrolyte solution led to creation of a U-wave that merged with the T-wave, indicative of an ionic imbalance. This is probably a consequence of acidosis, the acidosis is probably caused by the addition of a volume of approximately 50% the total blood volume of the animal. The addition of 5% glucose seems to counteract many of the adverse events, potentially due to activation of various glucose/ion co-transporter and symporter systems. Generally, the 5% glucose group maintained fewer adverse events and more normalized values than any of the other groups.

Dog Studies:

Study # 9-260: The single dose toxicity test of icodextrin by intraperitoneal administration in beagle dogs vol 1.8, p.26-90

Study # 5062: Dextrin 20 seven day intravenous toxicity study in dogs vol. 1.9, p. 1-57

Study # 7672: Dextrin 20 Peritoneal fluid exchange pilot feasiblity study in the beagle dog vol. 1.9, p. 58-151

Study # 7523: Dextrin 20 twenty-eight day peritoneal toxicity study in dogs vol. 1.9, p. 152-293, vol 1.10, p. 1-259

Study # 9-260: The single dose toxicity test of icodextrin by intraperitoneal administration in beagle dogs vol 1.8, p.26-90

This was a basic study to examine acute toxicity of a single dose of icodextrin solution via intraperitoneal injection in beagle dogs. There was no control group, 2 male and 2 female animals were in each group, and the total injection volume was 100 ml/kg (approximately 3x the human dose) with icodextrin

concentrations of 7.5, 13.5, and 25 % in the standard electrolyte solution (pH = 5.5). Dogs were followed for 14 days post-injection.

No deaths occurred in the study animals. All animals showed an increase in abdominal girth, probably due to water retention and flow into the peritoneal activity. Other significant early issues that resolved are: decreased locomotor activity, tachycardia, tremors, vomiting. Hematological and clinical chemical values were only collected 8-9 days prior to injection and one and two weeks after the injection. In all the animals, red blood cell numbers, hemoglobin and hematocrit dropped. In the 25% icodextrin group, White blood cells and neutrophils increased. No variation in clinical chemstry occurred in the 7.5% icdextrin group, in the 13.5% icodextrin group Albumin and A/G ratio declined while total cholesterol and ALP increased, and in the 25% icodextrin group Albumin and A/G ratio declined while total protein, total cholesterol, ALP and GPT increased.

The dosages used in this study were from 3 to 9 times the projected human dosage (projected to be 33.3 ml/kg of a 7.5% solution). Some effects were seen at all dose levels, although monitoring of hematological and clinical chemistry values were only done 7 and 14 days post-dosing. This probably implies a low safety factor, however, with volume and concentration controlled, it would be difficult to overdose on this treatment.

Study # 5062: Dextrin 20 seven day intravenous toxicity study in dogs vol. 1.9, p. 1-57

3.1 DOG 7-day IV Repeat Dose Pilot Study

The purpose of this study was to determine tolerability of icodextrin solutions at concentrations of 2.5%, 10% and 20% infused iv to 2 conscious beagles (1M, 1F) over a 4 hr daily period in sequence for each of 2 consecutive days at 10 ml/kg/h for 4 hrs for 7 days. The total daily doses were ≈ 1, 4 or ≈8 g/day, respectively , per dog.

The following chart, provided by drug sponsor, is an overview of the study design.

Subchronic Toxicity - Pilot - 7 Day - Beagle Dog

Name of finished product ICODEXTRIN 7.5%		Name of active ingredient ICODEXTRIN								
REPEATED DOSE TOXICITY Subchronic toxicity (up to 3 months)										
Ref. to document: Section 8, Attachment 13 Report date: Project Number: — 637361 Rept. No. 5062 Study period 1987.										
Species/Strain: DOG/BEAGLE										
Number of animals: 2			Duration of treatment: 7 DAYS							
Observation period after the end of dosing: NONE										
Administration route I.V. INFUSION INTO CEPHALIC VEIN										
Treatment of controls: NO CONTROL			Age: NOT STATED at study Body weight. 10-11.2KG initiation Treatment days per week: 7							
Study group	CONTR		DAY 1-2		DAY 3-4		DAY 5-7			
Dosage <TOTAL (G) PER DAY >	0		11	9.3	44	37.6	88	75.2		
Sex (m/f)	m	f	m	f	m	f	m	f		
Number of test animals	0	0	1	1	1	1	1	1		
Number of animals died or sacrificed in extremis			0	0	0	0	0	0		
Clinical observations: yes			Clin. chemistry: yes							
Food consumption: yes			Urinalysis: yes							
Water consumption: no			Organ weights: yes							
Body weight: yes			Necropsy: yes							
Hematology: yes			Histology: yes							
Additional examinations: ECG, DAILY BLOOD SAMPLES FOR CARBOHYDRATE ANALYSIS										
Additional information: TEST MATERIAL INFUSED FOR 4 HOURS PER DAY, AT 10 ML.KG^{-1}. H^{-1}. RISING DOSE STUDY										

Results

ECG tracings (Lead II) showed no abnormalities as a result of iv infusion of icodextrin solutions. Changes in heart rate reflected agitation during the measurement.

Polyuria, increase in plasma Na+ and small decrease in K+, creatinine and glycosuria were reported over the 7-day dosing period. The only histopathologic changes reported consisted in vacuolation (not severe) of hepatocytes. Drug sponsor concluded the 2 beagles in this study tolerate iv administration of increasing doses of icodextrin sol. (2.5 up to 20%) with no remarkable signs of toxicity.

2 beagle dogs, one male, one female; received 4 hour daily infusions of icodextrin solution in normal saline. Concentrations were 2.5, 10 and 20% icodextrin, with animals receiving 2.5% on days 1-2, 10% on days 3-4, and 20% on days 5-7. Animals were sacrificed on day 8 and necropsied. Blood samples were collected on a daily basis and ECG's were done.

No deaths occurred in the study. There were no severe reactions or changes to various clinical parameters in the study. Only some abnormal vacuolation of the liver occurred, and probably a longer study would be needed to determine if the observation was meaningful.

This study, can probably be taken as an indication that icodextrin itself, as opposed to in conjunction with the PD-2 electrolyte solution, is fairly non-toxic. The results also show that the icodextrin is broken down into glucose, and is a factor in glucose loads.

Study # 7672: Dextrin 20 Peritoneal fluid exchange pilot feasiblity study in the beagle dog vol. 1.9, p. 58-151

The objective of this study was to investigate the effects of prolonged BID exposure of the peritoneal cavity to icodextrin solutions.

The sponsor provided a detailed description of the surgical procedures for implantation of the PD catheter used in the beagles for administration and removing of the icodextrin solutions tested.

No histology was scheduled for this study.

The following table, provided by drug sponsor, is an overview of the study design.

Subchronic Toxicity - Pilot - 28 Day - Beagle Dog

Name of finished product ICODEXTRIN 7.5%		Name of active ingredient ICODEXTRIN			
REPEATED DOSE TOXICITY Subchronic toxicity (up to 3 months)					
Ref. to document: Section 8, Attachment 14 Report date: Dec 1991 Project No: — 641891 Report No: 7677 Study period (years): 1990					
Species/Strain: DOG/BEAGLE					
Number of animals: 6 (4M + 2F)			Duration of treatment: VARIABLE 8-29 DAYS		
Observation period after the end of dosing: NONE					
Administration route INTRAPERITONEAL					
Treatment of controls: NO CONTROL GROUP			Age: 4.5-6 MONTHS at study initiation Body weight: 9.5 - 11.9KG Treatment days per week: 7		
Study group *					
Dosage *	0		*		
Sex (m/f)	m	f	m	f	
Number of test animals			4	2	
Number of animals died or sacrificed in extremis			0	0	
Clinical observations: yes			Clin. chemistry: yes		
Food consumption: yes			Urinalysis: yes		
Water consumption: yes			Organ weights: no		
Body weight: yes			Necropsy: yes no		
Hematology: no			Histology: no		
Additional examinations: PERITONEAL FLUID					

* Batch nos. for icodextrin: at 14% 90C21BF; at 20% 90C22BF.

Peritoneal Fluid Exchange Feasibility Study in Beagle: Schematic Diagram to Illustrate the Animal Model.

Dextrin Polymer
Peritoneal Fluid Exchange Pilot Feasibility Study in the Beagle Dog
Schematic Diagram to Illustrate Experimental Model

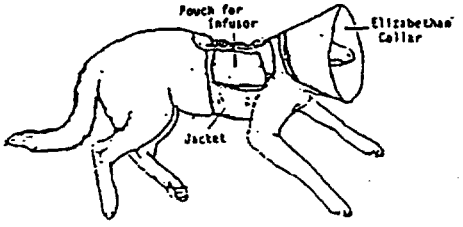
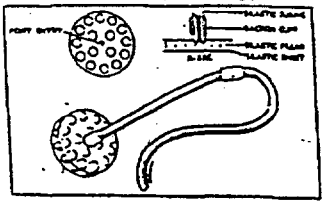
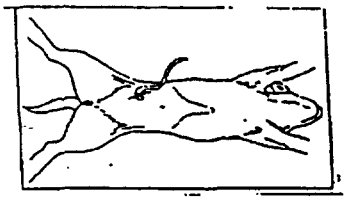


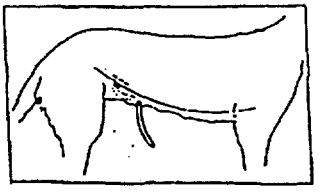
Illustration of Dog Jacket, Infusor Pouch and Elizabethan Collar Set-up



'Immobilised disk' catheter with double-layered disk and separative pillars



Position of catheter in abdominal cavity



Position of catheter for fluid administration/recovery

Results:

Water consumption was increased by dogs treated with icodextrin. Food intake was variable and caused fluctuation in body weight during the study; icodextrin treated dogs showed a dose-related decrease in food intake. Polydipsia and polyuria was reported for the glucose and icodextrin in some treated beagles.

Urinalysis of all dogs showed white blood cells and blood pigments.

Icodextrin treated dogs showed a decrease in BUN and creatinine when compared to the treatments.

Peritoneal fluid was recovered from some dogs; drug sponsor did not consider that sufficient dogs provided the fluid to permit reliable assessment of changes noted in protein and WBC counts in fluid.

Necropsy showed reddening of some tissues in control and drug treated beagles (i.e., intestine, omentum, pancreas. The catheter in the peritoneum was usually surrounded by omentum.)

Remarkable histopathologic changes reported included scars, some fibrosis around the implanted catheter.

Drug sponsor concluded peritoneal administration of icodextrin solutions to beagles via an indwelling catheter was a viable test model for repeated ip administration at high exposure levels of the test materials. The sponsor asserted that the MTD in dog was a 20% icodextrin solution.

This study was primarily designed to test the feasibility of placing a catheter into beagle dogs and carrying out a peritoneal fluid exchange. Fluid was exchanged twice daily at a dose of approximately 30 ml/kg of either electrolyte solution or 5% glucose in electrolyte solution or 14 or 20% icodextrin in electrolyte solution.

Interpreting the results of this study are difficult. Of the 6 animals, only animals 4 and 5 completed the study while on the same treatment, animal 2 completed the study, but was on three different treatments. Animals 1 and 6 were only in the study 8 and 11 days, respectively. The reviewer will therefore agree with the study authors that this study demonstrated that catheter use and fluid exchange is reasonable in a study.

Study # 7523: Dextrin 20 twenty eight day peritoneal toxicity study in dogs vol. 1.9, p. 152-293, vol 1.10, p. 1-259

The purpose of this definitive study was to investigate the systemic toxicity of icodextrin solutions injected in beagles (with surgically implanted peritoneal dialysis catheters) following repeat twice daily peritoneal exchanges for 28 consecutive days. Local tolerance and possible accumulation within cells of the reticulo-endothelial system was also studied.

An assay was developed to separate and analyses the components of glucose polymer in plasma, dialysis and urine samples. The assay is described (precision and accuracy also reported) in the submission.

The following table provided by drug sponsor gives an overview of the study design.

REPEATED DOSE TOXICITY Subchronic toxicity (up to 3 months)								
Ref. to document: Section 8, Attachment 15 Report date:JUNE 91 Project Number: — 641933 Rept. No. 7523 Study period (years): 1990 .								
Species/Strain: DOG/BEAGLE								
Number of animals: 30			Duration of treatment: 28 DAYS					
Observation period after the end of dosing: 14 DAYS, RECOVERY GROUPS 1,3,4.								
Administration route INTRAPERITONEAL								
Treatment of controls: I.P. INSTILLATION OF ELECTROLYTE SOLUTION			Age: 5 - 5.5 MONTHS at study initiation Body weight:7.3 - 11.5 KG Treatment days per week: 7					
Study group	(1) Control		(2) Glucose		(3) Icodextrin		(4) Icodextrin	
Dosage < G/KG/BD >	0		1.5		4.2		6.0	
Sex (m/f)	m	f	m	f	m	f	m	f
Number of test animals	3	3	3	3	3	3	3	3
Number of animals died or sacrificed in extremis	0	0	0	0	0	0	0	0
Clinical observations:	yes		Clin. chemistry:		yes			
Food consumption:	yes		Urinalysis:		yes			
Water consumption:	yes		Organ weights:		yes			
Body weight:	yes		Necropsy:		yes			
Hematology:	yes		Histology:		yes			
Additional examinations: PERITONEAL FLUID, ELECTROCARDIOGRAPHY, OPHTHALMOSCOPY, BLOOD LEVEL AND EXCRETION STUDIES, FECAL ANALYSIS FOR OCCULT BLOOD, ELECTRON MICROSCOPY OF LIVER SPLEEN AND LYMPH NODE								

Results

In this study, investigators treated the dogs with antibiotics, orally hydrate them with electrolyte solutions, and the icodextrin treated dogs were occassionally fed with liquid dietary supplements to insure their well-being and continue the study.

Drug sponsor stated that the dosage volumes administered were relatively consistent for each dog throughout the study.

Remarkable signs reported in the icodextrin treated dogs during the 28-day dosing period consisted of abdominal distension, and inappetence (also noted in several dogs from other groups.) Transient changes in body weight were reported for some dogs.

IND ~ 23

Compared to control, food consumption was reduced in dogs treated with glucose and icodextrin solutions.

An increase in water consumption was recorded for icodextrin and glucose treated dogs when compared to controls.

A reduction in urinary volume, pH, Na^+, and increase in specific gravity, K^+, creatinine output were reported for icodextrin treated dogs. Creatinine clearance rates were also lightly increase in icodextrin treated dogs towards the end of the study.

Clinical chemistry presented increased glucose levels in HD icodextrin dogs; the increased achieved statistical significance only some days; glucose levels were significantly increased for the LD at the end of the study.

At day 29 of study, clinical chemistry (group mean of M and F combined) showed no remarkable changes except for a dose-related increases in blood Cl- in icodextrin treated dogs; other changes included increase in plasma glucose (up to ~30% at HD icodextrin)

Increases in alkaline phosphatase were reported for icodextrin treated dogs; these were statistically significant increased ($p<0.01$) in the F dogs. Other changes reported included decreases in albumin, albumin/globulin ratio and calcium levels; some of these changes were significant in the icodextrin treated F dogs.

At necropsy, several findings,i.e., red areas in the mesentery, abdomen and intestine, where considered related to the peritoneal disk catheter or ip administration; no gross pathologic changes were reported to be related to the test materials.

Autopsy revealed in some dogs fibrosis around the peritoneal catheter; other changes reported red areas in the mesentery, abdominal wall and intestines and adhesions in the mesentery/intestines. Adhesions were reported at terminal kill: Group 1 Fs only (1 F after 4 wks of treatment- mesentery adhesions to left ventral wall; 1 F after 7 wks of treatment- adhesions between loops of small intestine), and in Group 3 (1 F after 4 weeks of treatment adhesions between loops of small intestines) and 1 M after 7 weeks of treatment mesentery adhesions to abdominal wall.)

Only adrenal gland weight increase was reported in the HD icodextrin group.

Histologic findings showed vacuolated and hyperplastic zona glomerulosa was noted in a few dogs treated with icodextrin; this findings was considered by drug sponsor to be a physiologic response perhaps due to increase aldosterone production in attempt to conserve sodium, which may possibly have passed from the blood into the icodextrin peritoneal fluid.

IND [redacted] 24

♦ Recovery Period Study.

After completion of ≈28 days of treatment and at the end of the 2-week off-test period, all dogs were killed and subjected to detailed gross/histopathologic evaluation and organ weight analysis.

Subchronic Toxicity - Formal - 28 Day - Beagle Dog

Name of finished product ICODEXTRIN 7.5%		Name of active ingredient ICODEXTRIN					
REPEATED DOSE TOXICITY Subchronic toxicity (up to 3 months)							
Ref. to document: Section B, Attachment 15 Report date: JUNE 1991 Project Number: 641933. Rept. No: 7523 Study period (years) 1990.							
Species/Strain: DOG/BEAGLE							
Number of animals:30			Duration of treatment:28 DAYS				
Observation period after the end of dosing: 14 DAYS, GROUPS 1,3,4.							
Administration route INTRAPERITONEAL							
Treatment of controls: I.P. INSTILLATION OF ELECTROLYTE SOLUTION			Age:5-5.5 MONTHS Body weight: 7.3 - 11.5 KG at study initiation				
			Treatment days per week: 7				
Study group	(1) Control Recovery		(3) Recovery		(4)Recovery		
Dosage <G/KG/BD>	0		4.2		6.0		
Sex (m/f)	m	f	m	f	m	f	
Number of test animals	1	1	1	1	1	1	
Number of animals died or sacrificed in extremis	0	0	0	0	0	0	
Clinical observations:	yes		Clin. chemistry:		yes		
Food consumption:	yes		Urinalysis:		yes		
Water consumption:	yes		Organ weights:		yes		
Body weight:	yes		Necropsy:		yes		
Hematology:	yes		Histology:		yes		
Additional examinations ELECTROCARDIOGRAPHY, OPHTHALMOSCOPY							

Results

Drug sponsor stated that the recovery dogs showed evidence of the histologic changes noted during the dosing period, but to a lesser extent and were considered to be returning to normal.

The drug sponsor concluded that the results of these studies indicate that peritoneal administration of 14% and 20% icodextrin to beagles for 28 days produces changes which are probably evidence of physiologic reactions induced by the osmotic properties of the icodextrin solutions.

Further observations:

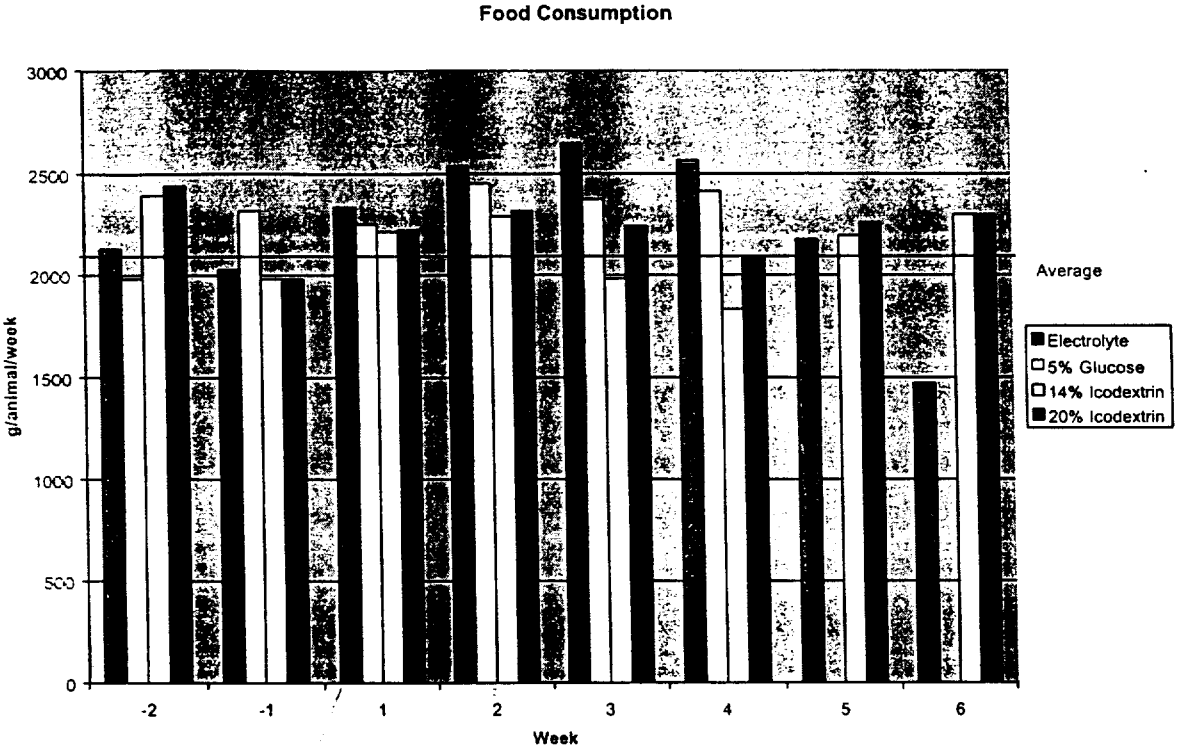
TABLE 1

Dextrin Polymer
28 Day Peritoneal Toxicity Study in Dogs
Incidence of Clinical Signs

Group/Treatment (30 ml.kg^{-1} b.d.)	Signs Observed	
	Abdomen Distended	Inappetence
1 Electrolyte Solution	0	13
2 5% Glucose	17	23
3 14% Dextrin Polymer	31	50
4 20% Dextrin Polymer	47	51

Figures given indicate number of times observation recorded per group over the course of the 28 day dosing period

Results from the study showed that during the study, the dogs receiving icodextrin treatment displayed more clinical signs than those in the glucose or solution groups.



Food consumption was not affected by treatment except with the electrolyte treated in the 6th week (2nd recovery week). There was a concomitant drop in body weight in the male in the recovery group, and in all the females in the recovery group. No reason was given for the rapid weight loss in the female dogs.

FIGURE 2

Dextrin Polymer
28 Day Peritoneal Toxicity Study in Dogs
Body Weight : Males

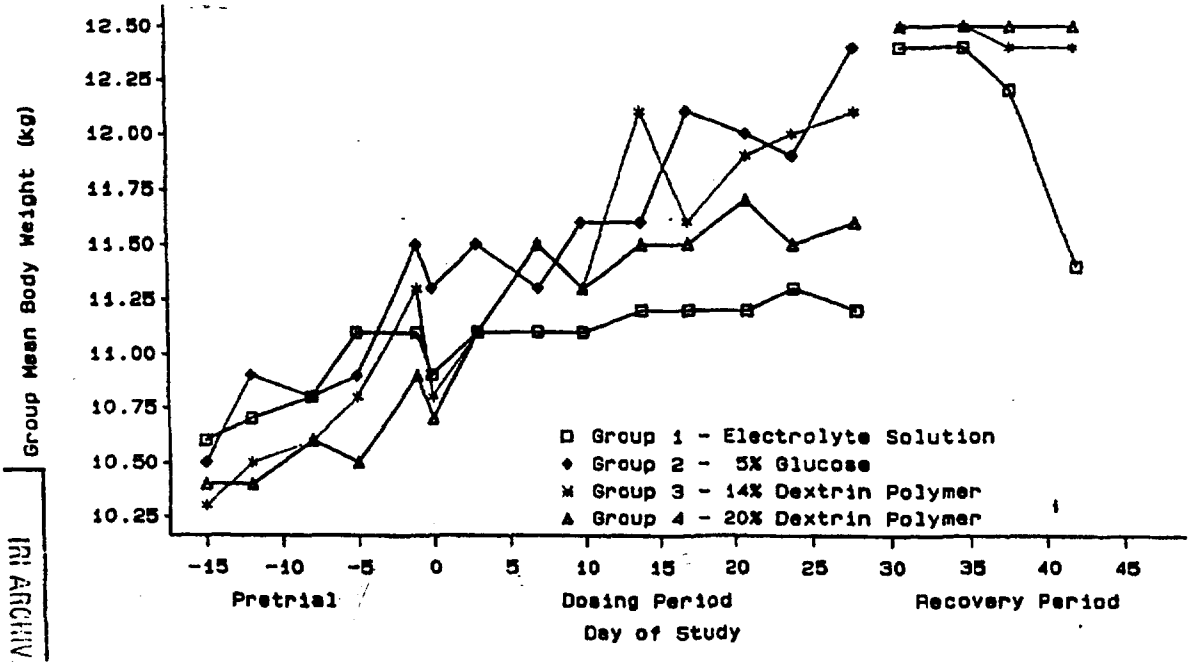
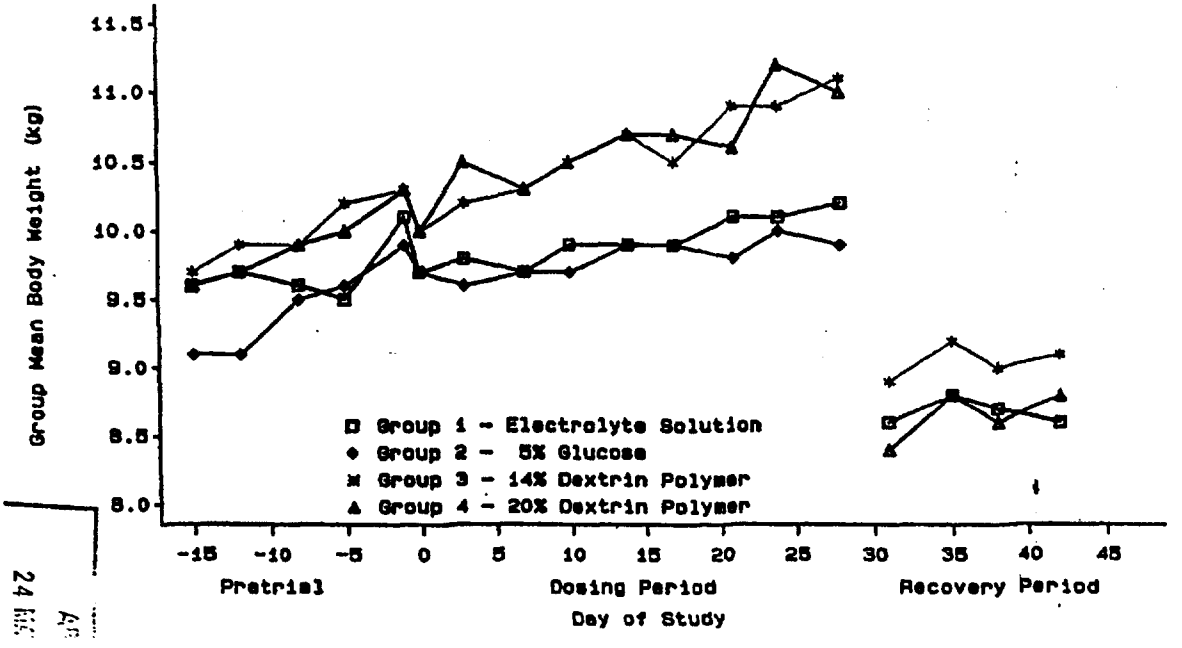


FIGURE 3

Dextrin Polymer
28 Day Peritoneal Toxicity Study in Dogs
Body Weight : Females



Necropsy results raised a number of concerns for the study population. Fatty deposits in the inner cortex of the kidney may indicate a developing nephrotic syndrome. This, coupled with the reduced excretion of sodium and the increase in urinary creatinine and creatinine clearance are probably indicative of developing kidney problems.

Several clinically significant changes occur in the clinical chemistry. Alkaline phosphatase levels were elevated in all the groups, but especially in the 14% and 20% icodextrin treated animals. This trend moderates, but remains elevated in the recovery groups. Interestingly, LDH increase dramatically in the recovery period, potentially indicating cell damage. Potassium levels are low in all except the 20 % icodextrin group, and phosphorus values are below the clinical reference range. Glucose values are normal for the electrolyte and 5% glucose groups, but is elevated in the 14 and 20 % icodextrin groups.

Of concern too is the development of serosal reactions, potentially indicative of an allergic response, in the peritoneum. Only 6 of the 16 animals not treated with icodextrin exhibited any sign of a serosal reaction in the peritoneum, and those were equivocal (grade +/-), while in the icodextrin treated animals all exhibited signs of a serosal reaction, with several reaching a grade +++ strong reaction. Since there are reports of humans developing allergic symptoms to icodextrin, perhaps this would be a system to further examine the problem of icodextrin allergies.

Water balance is very positive (see dog pk study). Probably most of the weight gain is due to edema, since food intake drops, particularly in the 20% icodextrin group. With regard to measures of the organ weights, we have no way of knowing if any of the organs for the organ weights are edematous, making the use of wet organ weights problematic at best. Wet and dry weights should probably have been reported. In addition, although urinary sodium decreases to almost nothing in the high dose icodextrin group, there is no increase in plasma sodium levels, in fact they drop lower, although still within the physiological range. A major question is "What happened to the excess sodium?"

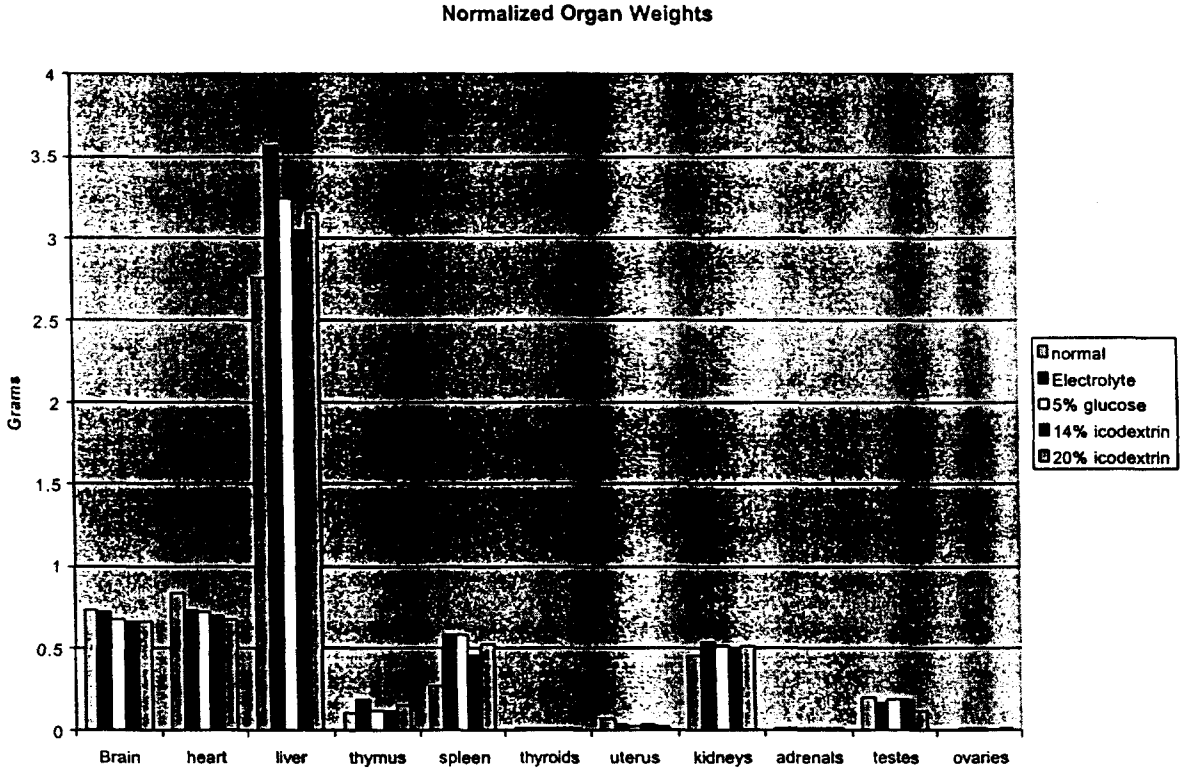
Short term studies do not allow for assessment of how much damage is occurring to the kidneys, and whether the glycogen deposits in the liver would eventually lead to liver problems. Hepatotoxicity and liver problems are frequently co-morbid conditions in peritoneal dialysis.

The peritoneal fluid was not assessed for electrolyte levels or carbohydrates. This information is important. Additionally, the 5% glucose seems to moderate the effects of the electrolyte solution better than the 14 and 20% icodextrin solutions for some effects.

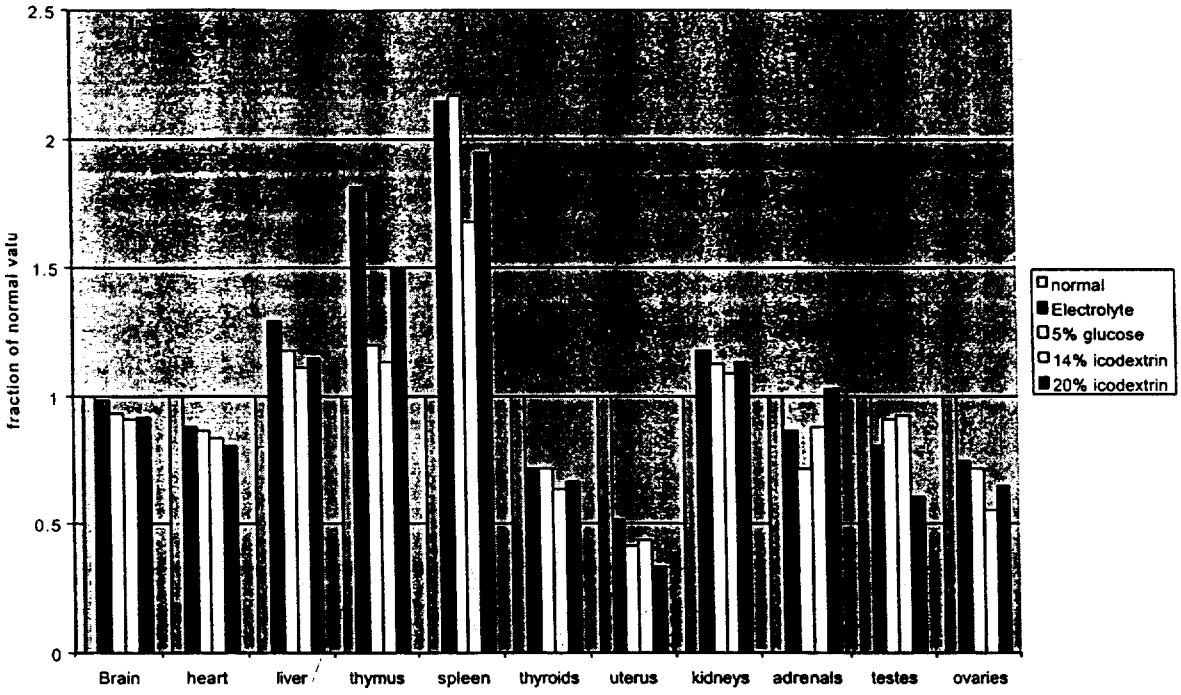
A very high percentage (100% in the electrolyte, 5% glucose, and 20% icodextrin; 33%-1 of 3- in the 14% icodextrin group; 12 of 14 treated animals) had positive findings in the testes, most being immature, a very unusual finding for 6-6.5 month old dogs.

Isosthenuria occurs in the electrolyte & glucose group during the treatment phase, and during the recovery phase in all groups, indicating a problem in the ability of the kidneys to concentrate urine. Sodium excretion essentially stops in the 20% icodextrin group, with a reduction in the 14% icodextrin group. This provides further evidence of kidney damage, probably a longer term study would better show this result.

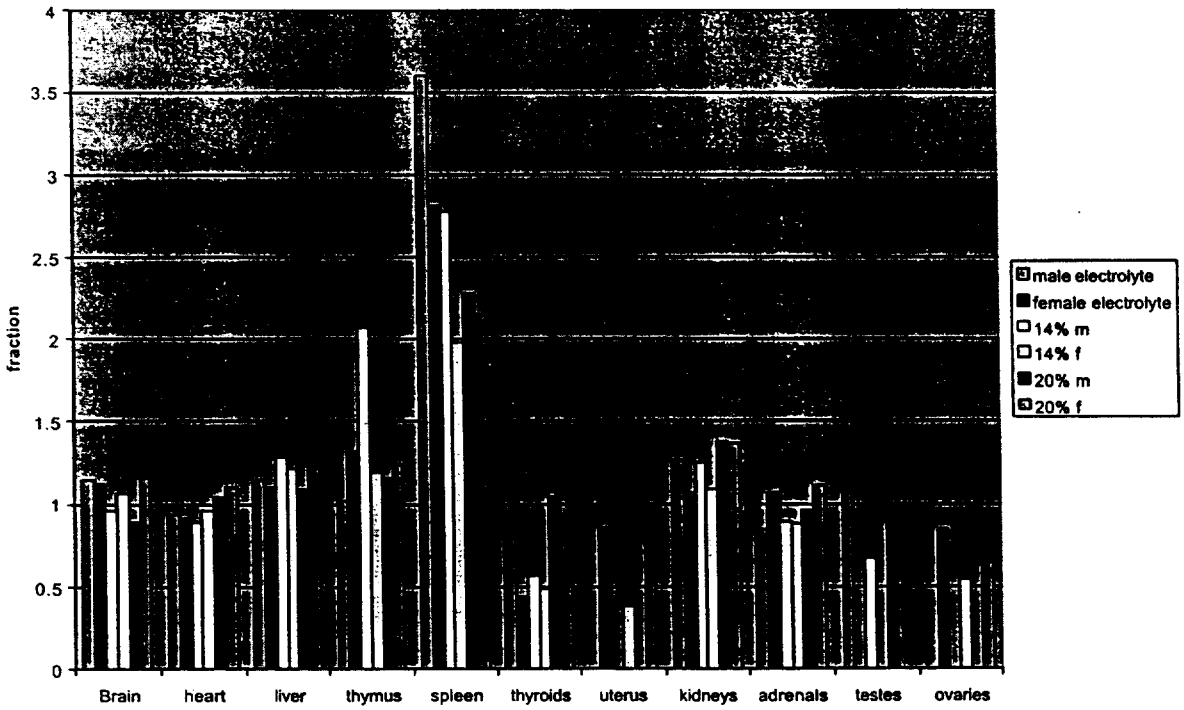
Organ weight studies increased concerns about the treatments studied here. Additionally, unlike in the rat toxicity study, animals did not recover during the 14 day recovery period, and some conditions were exacerbated.



Dog Organ Weights - relative to normal



recovery dogs - fraction of normal values



Prominent splenomegaly, declines in uterine and ovarian tissue, as well as thyroids occur. These trends continue strongly into the recovery phase. Damage may be permanent.

Deficiencies:

No normal values provided or information on clinical chemistry analyzer used

Issues:

Gender differences

Kidney effects

Liver effects

Alkaline phosphatase, phosphorus,

Toxicology summary: Unfortunately, all the toxicology studies use the electrolyte solution for a control with the exception of one blood volume and electrolyte study. This study demonstrated that the electrolyte solution, used widely as a control throughout the NDA, is not benign, but leads to changes in blood volume and electrolytes. The toxicology studies further bear this out, with the electrolyte solution alone leading to heart, kidney and liver damage and damaging the testes in rats. Since this electrolyte solution is the vehicle for icodextrin peritoneal dialysis, it is very germane to the effects seen here.

The major 28 day repeat dose dog and rat studies also differ on one key point. The rat study injects a large volume of fluid on a daily basis without fluid withdrawl, while the dog study is using a peritoneal dialysis set up. The major concern stems from the further kidney damage caused by the electrolyte solution, especially in the dog model system. A significant part of the problem may be due to the pH of the electrolyte solution. By adding a large volume, approximately half the total blood volume, of a pH 5.5 solution to the peritoneal cavity is probably creating an acidotic environment. Among the consequences of acidosis are electrolyte problems, kidney and liver damage, and U-waves on the ECG, all of which are seen here. Because of the use of the electrolyte solution as a control, it is not realistic to assess the toxicity of icodextrin.

Toxicology conclusions: Icodextrin is probably of minimal toxicity, however, the results presented here make it difficult to draw such a conclusion. This drug evaluation is for the entire formulation, including the PD-2 electrolytes. What is needed are 1) appropriately controlled studies, 2) longer, chronic toxicity studies to assess longer term damage (i.e. kidney, liver and potential lung toxicity), 3) further study of gender differences in peritoneal dialysis.

Histopathology Inventory for NDA #

Study	7423	7523
Species	CD rats	Beagles
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)		
Brain	X*	X*
Cecum	X	X
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis		X
Esophagus	X	X
Eye	X	X
Fallopian tube	X	X
Gall bladder	X*	X*
Gross lesions	X	X
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		
Larynx		
Liver	X*	X*
Lungs	X*	X*
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves		X
Ovaries	X*	X*
Pancreas	X	X*
Parathyroid		
Peripheral nerve		

Pharynx		
Pituitary	X*	X*
Prostate		
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles		
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina		
Zymbal gland		
Standard List		

X, histopathology performed
*, organ weight obtained

GENETIC TOXICOLOGY: 3 Studies were done to examine the mutagenicity of icodextrin. There was no indication from any of the studies of mutagenic potential with icodextrin treatment.

Studies performed:

Dextrin 20 powder, Batch no. QC001/B: Testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA98, and TA100.

Dextrin 20 powder: Chromosomal aberrations assay with Chinese Hamster Ovary cells in vitro.

Dextrin 20 powder: Micronucleus test in bone marrow of CD-1 mice.

Study title: Dextrin 20 powder, Batch no. QC001/B: Testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA98, and TA100.

Insert abstract

Key findings:
Study no: 6814
Study type (if not reflected in title):
Volume #, and page #: vol. 11, pp. 49-97
Conducting laboratory and location:
Date of study initiation: November 2, 1990
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Dextrin 20 powder, lot # QC001/B
Formulation/vehicle: sterile ultra-pure water

Methods:

Strains/species/cell line: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA98, and TA100
Dose selection criteria:
Basis of dose selection:
Range finding studies:
Test agent stability:
Metabolic activation system: S9 mix
Controls:
Vehicle:
Negative controls:
Positive controls:

Comments:
Exposure conditions:
Incubation and sampling times:
Doses used in definitive study:
Study design:
Analysis:
No. of replicates:
Counting method:
Criteria for positive results:

Summary of individual study findings:

1. Bacterial Reverse Mutation Assay (Ames test.) Report No. 6814 in v.6)

Briefly, reverse mutations assays were carried out using *S.* tiphimurium strains TA98, TA100, TA1535 and TA1537. Positive controls substances used were 2-aminoanthracene (2-ANN), sodium azide (NaN3), and 2-nitrofluorene (2-NF); these were dissolved in ultra-pure water. The male Fischer rat (previously injected ip with 500 mg/kg Aroclor 1254) liver microsome fraction (S-9) and co-factors were used as metabolic activator. Icodextrin was tested in duplicate assays at 6 concentration levels ranging from 33 up to 10000 µg/agar plate. (NB. ICH Guidelines states that the upper treament levels are 5000 µg/plate for fully soluble compounds. Icodextrin is freely soluble in water at 25° C.)

After agar plates were prepared with icodextrin or positive/negative controls, the bacterial solutions, with/without S-9 were incubated at 37°C for 2 days. After this time, the reverse mutation colonies occurring on a plate were counted. A mutagenic response was recorded if for strains TA98, TA1535, TA1537 and TA1538 the average number of reverse mutation colonies were double, and for TA100 1.5 times, the average number of reverse mutation colonies the average concurrent negative control values; a dose-related response, and a reproducible effect in independent tests.

Results

Drug sponsor reported that no toxicity to the bacteria or precipitation of the test material was noted. Vehicle control values were within the normal ranges of investigators' laboratories. Results obtained in the positive control groups were within the normal ranges. For tests rejected due to plate contamination (TA 1538 with/without S9 mix), repeat tests were performed and all tests were acceptable according to the study criteria.

In the definitive studies, there was no increase in the number of reverse mutation colonies in icodextrin (with/without S9 mix) treated cultures relative to negative controls. Concurrent positive controls demonstrated sensitivity of the assay and the metabolizing activity of the S-9 mix with results within the normal ranges expected for each bacterial strain.

Study validity:
Study outcome:

Genetic toxicology summary:

Genetic toxicology conclusions:

Labeling recommendations:

Summary of individual study findings:
Study validity:

Study outcome:

Study title: Dextrin 20 powder: Chromosomal aberrations assay with Chinese Hamster Ovary cells in vitro.

Summary:

From E. Barry IND —— Review:

2. In Vitro Cytogenetics (Chromosomal aberration assay.) Report No. 8263 in v. 6.

Briefly, chromosomal aberration tests were carried out in the presence/absence of S-9 mix from livers of Aroclor 1254-induced adult male rats using the Chinese hamster ovary (CHO) cell line to determine clastogenic potential of icodextrin. The vehicle used for compounds tested was culture media.

Cyclophosphamide (CPH was S-9 activated) and methyl methanesulphonate (MMS was not S-9 activated) served as positive controls in the assay.

Toxicity tests were performed to establish the range of icodextrin concentrations to be used in the main chromosomal aberrations assay. Nine concentrations of icodextrin (ranging from 15 up to 150000 µg/ml in culture flask) were used. In the main chromosomal aberration assay (duplicate cell cultures), concentration selected were: 4 icodextrin concentrations (25000 up to 200000 µg/ml) in the presence of S-9, and 5 concentrations of icodextrin (75 up to 200000ug/ml) in the absence of S-9 mix. Treatment was for 6 hrs in the presence of S-9 and for 24 hrs in the absence of S-9 mix.

Chromosome preparations were made as follows: Colcemid (0.01 µg/ml) was added to the culture 2 hr before harvesting. Mitotic cells were recovered by decanting into tubes. Monolayer cells were recovered by trypsinization, counted and discarded.

Concurrent positive and negative controls were run to test the sensitivity of the system.

Evaluation criteria for the definitive test was fully described in the submission (i.e., chromatid/chromosome gaps, breaks, acentric fragments, metaphases with chromosome with multiple aberrations, etc.)

Results

Drug sponsor reported that in the toxicity test icodextrin (with/without S-9 mix) was non precipitating and non toxic at all concentrations tested.

In the definitive assay, there was no evidence that icodextrin induced chromosomal aberrations in the presence/absence of S9 mix.

Drug sponsor concluded that icodextrin was not clastogenic when tested in vitro with CHO cells.

Study no: 8263
Study type (if not reflected in title)**:**
Volume #, and page #: vol. 11, pp. 98-136
Conducting laboratory and location: [redacted]
Date of study initiation: June 25, 1991

GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Dextrin 20 powder, lot # DX045/C
Formulation/vehicle: Ham's F10 Cell culture media

Methods:

Strains/species/cell line: Chinese Hamster Ovary cells (CHO-10 B_4)
Dose selection criteria:
Basis of dose selection:
Range finding studies:
Test agent stability:
Metabolic activation system: S9 mix
Controls: Ham's F10 media

Positive controls: cyclophosphamide with S9 mix, methyl methanesulphonate without S9
Comments:
Exposure conditions:
Incubation and sampling times:
Doses used in definitive study:
Study design:
Analysis:
No. of replicates:
Counting method:
Criteria for positive results:

Summary of individual study findings:
Study validity:
Study outcome:

Genetic toxicology summary:

Genetic toxicology conclusions:

Labeling recommendations:

Summary of individual study findings:
Study validity:
Study outcome:

Study title: Dextrin 20 powder: Micronucleus test in bone marrow of CD-1 mice.

Summary:

Key findings:

Study no: 8468
Study type (if not reflected in title):
Volume #, and page #: vol. 11, pp. 137-168
Conducting laboratory and location: .
Date of study initiation: December 11. 1991
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Dextrin 20 powder, lot # DX045/C
Formulation/vehicle: distilled water

Methods:

Strains/species/cell line: CD-1 mice
Dose selection criteria:
Basis of dose selection: previous study (— project # 751566) determined maximum acheivable dose
Range finding studies: 5, 10 and 20 % icodextrin in distilled water given at a dose of 30 ml/kg/animal
Test agent stability: stable
Metabolic activation system:
Controls: distilled water

Positive controls: cyclophosphamide

Summary of individual study findings: (From E. Barry Review)

3. Mouse (CD-1) micronucleus test. Report No. 8468.

In this test, 6 groups of mice/sex were dosed by ip route once (at 30 ml/kg) with icodextrin solutions (20 mice/sex at 5%, 10% and 20% and a satellite group of 5 mice/sex with 20% icodextrin), distilled water (15 mice/sex) and cyclophosphamide (15 mice/sex at 80 mg/kg in 10 ml/kg). The doses of icodextrin were selected based on the maximum achievable dose to get into solution- 20%

Peripheral blood samples were taken from the 5 mice/sex treated with 20% icodextrin, and bone marrow samples from 5 mice/sex at 24h, 48h and 72h after treatment with positive/negative controls and 20% icodextrin; bone marrow samples were taken at 24 hr from mice treated with 5% and 10% icodextrin solution.

Blood and bone marrow sample preparations were fully explained in the submission. Briefly, blood samples via cardiac bleed were taken 2h after treatment from satellite group. Bone marrow samples, taken from mice that were killed, were taken from femur and treated with hypotonic solution to make micronuclei visible. After centrifugation and supernatant fluid was discarded, the cells were resuspended and a drop of the suspension was used to prepare slides. Two slides, from each mice, were prepared. Slides were rinsed in distilled water/air dried and prepared for microscopic reading. One thousand polychromatic erythrocytes (PCE) per mouse were scored for micronuclei. To avoid artifacts, the number of micronucleated normochromatic erythrocytes (NCE) were also recorded. The PCE/NCE ratio was determined for each mouse by counting the number of immature PCE per mature NCE erythrocytes in a minimum total of 500 cells (PCE + NCE).

Results

Drug sponsor reported that no micronucleus induction was detected in bone marrow erythrocytes of mice treated with the 3 solutions of icodextrin or distilled water. The cyclophosphamide treated groups demonstrated increases in the frequencies of bone marrow micronuclei when compared to other sampling times. Mice treated with distilled water alone showed normal background levels of micronuclei.

Drug sponsor concluded that icodextrin was devoid of micronucleus inducing potential in bone marrow of CD-1 mice when tested to maximum achievable doses using a single ip exposure and multiple sampling regimen.

Genetic toxicology summary:

Genetic toxicology conclusions:

Labeling recommendations:

Genetic toxicology summary: No evidence of genetic toxicity by icodextrin

Genetic toxicology conclusions:

Labeling recommendations:

CARCINOGENICITY: NOT DONE

REPRODUCTIVE TOXICOLOGY:

Icodextrin combined study of effects on fertility and embryo-fetal toxicity in CD rats by intraperitoneal administration

This 8 week study utilized 196 virgin adult CD strain rats from
Males weighed between 299 and 247 g, females weighed between 201-241 g. Rats received a daily intraperitoneal injection of 10 ml/kg/day electrolyte solution (Controls), 5 ml/kg/day of a 20% Icodextrin/electrolyte solution (1g/kg icodextrin), 10 ml/kg/day of a 20% Icodextrin/electrolyte solution (2 g/kg icodextrin), or 20 ml/kg/day of a 20% Icodextrin/electrolyte solution (4 g/kg). The only exception to this protocol was that it was decided that 20 ml/kg/day was a large volume to inject intraperitoneally into pregnant rats, and this treatment was withheld from the females. These dosages are less than the 30 ml/kg of 7.5% icodextrin that is predicted to be the expected human dose (2.25 g/kg icodextrin), except for the very highest dose which was only 1.78 fold the human dose). The control solution was even much less than was generally used, where in the rat toxicology study animals received 30 ml/kg twice a day. Varying the volumes and changing the concentration of the icodextrin solution lessened the exposure to the electrolyte solution, which caused problems in the rat toxicology study.

The results were largely unremarkable. Generally fertility and fecundity were apparently not impaired by the treatments. Nor were sex ratios of fetuses significantly different among the treatments. The numbers of implantations and live fetuses were well within the parameters characteristic of this strain. The litters were harvested on day 20, and values recorded were within the typical litter sizes, patterns and weights for that gestation.

There was a higher incidence of diaphragmatic hernias, with the rate increasing from .026 to .086, more than 3-fold. However, these rates are both well within those of the historical controls. Similarly, although the number of uni- and bilateral supernumerary ribs is increased more than 2-fold with icodextrin, the values are less than those found in the historic controls. None of the fetal results indicated any potential problems.

The males were treated for 29 days before pairing until termination (approximately 7 weeks total), females for 15 days prior to mating until day 17 of gestation (approximately 4.5 weeks total).

In conclusion, the results of this study indicate no apparent effect of intraperitoneally administered icodextrin on fertility of fetal health in CD-Strain rats. However, this has to be interpreted carefully since the dosages used were less than those anticipated for humans. Additionally, in the rat toxicity studies, inflammation and tubular atrophy of the testes and epididymis were seen. Probably the lower doses prevented this from being seen in this study. Results from the dog toxicology study further decrease the utility of this study. In the beagles, almost all the animals had immature testes, and some had other problems (i.e. atrophy). It is not known if the immaturity was due to a developmental delay caused by the treatment, or a regression or damage caused by the treatment.

? on the testicular atrophy seen in the rat tox study?

MLB034/994906

SUMMARY TABLE

REPRODUCTION TOXICITY	ADMINISTRATION ROUTE: Intraperitoneal injection
COMBINED FERTILITY AND EMBRYO-FETAL TOXICITY (STUDY WITH CAESAREAN SECTION)	TREATMENT OF CONTROLS: Placebo SECTION ON FEMALES ON DAY 20
NAME OF COMPANY : ML Laboratories plc	STUDY PERIOD (Weeks): Eight
NAME OF FINISHED PRODUCT: Icodextrin (20% solution)	SPECIES / STRAIN: Rat/Sprague Dawley
REPORT NUMBER: MLB034/994906	NUMBER OF ANIMALS: 22 per sex per group

Sperm in vaginal smear = Day 0 of gestation
Treatment period for males: For 4 weeks before pairing continuously until termination
Treatment period for females: For 2 weeks before pairing continuously until Day 17 of gestation (volume dosed fixed on Day 6 of gestation)
* Group 4 - males only dosed: No detailed fetal examination performed.

METHODS OF FETAL EXAMINATION

Skeleton (✓) Histology (✗)	Soft tissue (✓) Macroscopic examination	Detailed visceral Examination (✓)

Group: Dose volume: (ml/kg/day)	1 10	2 5	3 10	4* 20
Adults males				
Confirmed fertile	22	22	22	22
Adult females				
Females with evidence of mating	22	22	22	22
Pregnant females	22	22	22	22
Evaluated pregnant females	22	22	22	22
Litters - group mean values				
Corpora lutea	16.8	15.9	16.4	16.1
Implantations	15.6	15.2	15.5	15.2
Live fetuses	14.1	13.8	14.5	14.5
Resorptions	1.5	1.5	1.0	0.7
Weight of fetuses (g)				
Males:	3.75	3.80	3.85	3.85
Females:	3.62	3.60	3.67	3.65
Sex ratios of fetuses (as % male)	51.1	57.0	51.1	48.5

IMPORTANT PARENTAL FINDINGS
No adverse effects.
IMPORTANT FETAL FINDINGS
None considered to be related to treatment.

Study conducted by the applicant: Yes ( ) No (✓)
If "No" indicate the name and address of the institute that conducted the study:

Study in compliance with GLP: Yes (✓) No ( ) Not required ( )

Report 99 4906 0008 002

Sponsor's Summary Table

MLB034/994906

SUMMARY

Procedures

This study was performed to investigate the influence of Icodextrin on the reproductive performance and fertility of male and female rats of the CD strain (of Sprague-Dawley origin), with particular reference to effects produced before pregnancy and during the initial and organogenesis stages of pregnancy. For this purpose, Icodextrin (20% solution) was administered by intraperitoneal injection at volume dosages of 5 or 10 ml/kg/day to groups of twenty-two sexually mature male and twenty-two sexually mature female rats. A further group of twenty-two sexually mature male rats received Icodextrin (20% solution) at a volume dosage of 20 ml/kg/day and were paired for mating with twenty-two untreated females. These volumes were considered to be the maximum practical under the conditions of this study; in the male, a daily volume of around 10 ml was achieved at 20 ml/kg/day by termination. In the female, a daily volume of around 3 ml was injected at 10 ml/kg/day between Day 6 and 17 of pregnancy.

Male rats were treated for 29 days before pairing, throughout pairing and until termination. Female rats were treated for 15 days prior to pairing, throughout pairing and up to and including Day 17 of gestation (volume dosed fixed on Day 6 of gestation). A Control group of twenty-two sexually mature male and twenty-two sexually mature female rats received the vehicle, an electrolyte solution, at a volume dosage of 10 ml/kg/day over the same treatment period.

Females were killed on Day 20 of gestation for examination of their uterine contents and the fetuses of treated and Control group females were subjected to detailed visceral and skeletal examinations. Males were killed after approximately seven weeks of treatment and the reproductive organs were weighed and preserved.

Results

There were no treatment-related mortalities.

Bodyweights and food consumption for the males were unaffected by treatment. For the females bodyweights and food consumption were unaffected throughout the pre-pairing and gestation periods.

Mating performance and fertility were similar in all groups.

Litter responses and detailed examination of fetuses at Day 20 of gestation suggested that there had been no adverse effects of treatment on embryo-fetal survival, growth or development.

Necropsy of parent females at Day 20 of gestation did not reveal any macroscopic findings considered to be related to treatment.

Necropsy of the parental males revealed slightly low epididymal weights in animals receiving 20 ml/kg/day compared with the Controls. The toxicological significance of this was unclear as no other reproductive organs were affected and all males were of proven fertility.

Conclusion

The evidence from this investigation demonstrated that 10 ml/kg/day of Icodextrin (20%) had no adverse effects on female condition, mating performance, fertility and embryo-fetal development. For the males 20 ml/kg/day of Icodextrin (20%) had no adverse effects on general condition, mating performance and fertility.

Sponsor's Summary

Study title: Icodextrin Conbined Study of Effects on Fertility and Embryo-Fetal Toxicity in CD Rats by Intraperitoneal Administration.

Key study findings: Study showed no effects of treatment on fertility in males or females at doses comparable to projected human exposure levels.

Study no.: MLB034/994906
Volume #, and page #: Volume 12, pages 1-169
Conducting laboratory and location: ———
Date of study initiation: May 28, 1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Icodextrin 20%, Batch # SP233222 and SP267238, Purity = 20.06%
Formulation/vehicle: 20% icodextrin in 40 mmol/L lactate, 0.25 mmol/L magnesium, 1.75 mmol/L calcium, 96 mmol/L chloride, 133 mmol/L sodium in purified water at pH 5.0-6.0. Control solutions were the same except without icodextrin.

Methods:

Species/strain: Rats/CD strain from ———
Doses employed:

Group	Treatment	Dose Volume (ml/kg/day)	Number of Animals		Animal Numbering	
			M	F	M	F
1	Control	10	22	22	1-22	89-110
2	Icodextrin	5	22	22	23-44	111-132
3	Icodextrin	10	22	22	45-66	133-154
4	Icodextrin	20	22*	22	67-88	155-176

Route of administration: Intraperitoneal
Parameters and endpoints evaluated: Clinical Signs, Body weights, food consumption and oestrous cycles were monitored along with mating procedure and time to mating. Terminal studies were
Males: testes, epididymides, prostate, seminal vesicles were weighed and retained at necropsy and placed in appropriate preservative solution.
Females: On Day 20 of gestation, females were killed and the reproductive tract dissected out and recorded for: number of corora lutea in each ovary, number of implantation sites, number of resorption sites, number and distribution of fetuses in each uterine horn.
Fetal Examinations:

Each fetus was weighed, sexed and examined for any external abnormalities. Individual placental weights were recorded and any placental abnormalities were noted. Fetuses were killed by chilling on a cool plate

The neck and the thoracic and abdominal cavities of approximately half of each litter were dissected and examined. Fetal changes were recorded and the offspring eviscerated prior to fixation in industrial methylated spirit. After fixation, fetuses were processed, stained with Alizarin Red and skeletal development assessed.

The remaining fetuses in each litter were placed in Bouin's fixative, subjected to free-hand serial sectioning and examined for visceral changes.

Fetuses from the untreated Group 4 females were not processed, but retained in Bouin's fixative or industrial methylated spirit. No data is, therefore, presented for these.

Results:

Mortality: None
Clinical signs:
Body weight:
Food consumption:
Toxicokinetics:

For fertility studies:
In-life observations:
Terminal and necroscopic evaluations:

OR

For embyrofetal development studies:
In-life observations:
Terminal and necroscopic evaluations:
Dams:
Offspring:

OR

For peri-postnatal development studies:
In-life observations:
Dams:
Offspring:

Terminal and necroscopic evaluations:
Dams:
Offspring:

Summary of individual study findings:

Reproductive toxicology summary: One concern from the general rat toxicology studies was that several of the rats in the control group 2/13 had testicular tubular atrophy and 7/13 had testicular inflammation. In the 20% icodextrin group 5/13 had testicular inflammation, while no adverse signs were seen in the 5% glucose or 14% icodextrin groups. One animal had a large green mass on the epididymus with no report on whether it was neoplastic.

In the beagle toxicity study, virtually all the dogs had findings of immature testes. This is unusual in the 6-7 month old 10 kg dogs used in this study. In addition, the ovaries and uterus were below normal sizes.

These results were not seen in the reproductive toxicology report. This is probably due to the dosing scheme reducing exposure to the PD-2 electrolyte solution and the generally lower doses of icodextrin, especially when compared to the toxicity studies or the projected human doses. Most of the other studies used a 30 – 40 ml/kg dosing scheme, the repro-tox study used 10 ml/kg of the electrolyte solution and as low as 5 ml/kg of the icodextrin containing solutions. Rats received a daily intraperitoneal injection of 10 ml/kg/day electrolyte solution (Controls), 5 ml/kg/day of a 20% Icodextrin/electrolyte solution (1g/kg icodextrin), 10 ml/kg/day of a 20% Icodextrin/electrolyte solution (2 g/kg icodextrin), or 20 ml/kg/day of a 20% Icodextrin/electrolyte solution (4 g/kg). The only exception to this protocol was that it was decided that 20 ml/kg/day was a large volume to inject intraperitoneally into pregnant rats, and this treatment was withheld from the females. These dosages are less than the 30 ml/kg of 7.5% icodextrin that is predicted to be the expected human dose (2.25 g/kg icodextrin), except for the very highest dose which was only 1.78 fold the human dose). Varying the volumes and changing the concentration of the icodextrin solution lessened the exposure to the electrolyte solution, which exhibited signs of toxicity in the rat toxicology study.

Reproductive toxicology conclusions:

Labeling recommendations:
No effects were seen in female animals in the reprotox study, however reductions in ovarian and uterine size were seen in the toxicity studies. In male rats, some testicular atrophy and inflammation was found. In male dogs, immature testes and some atrophy was seen. No effect on fertility was observed in the short-term studies reported, however, doses and exposure levels were much lower than that in other studies. Long-term studies were not done. Therefore, long-term consequences of extraneal therapy is not known. Sperm motility studies?

SPECIAL TOXICOLOGY STUDIES:

IMMUNO-TOXICOLOGY:

Study title: Study 10652: Evaluation of potential for icodextrin to induce anaphylaxis in guinea pigs.

Summary: This was a very typical guinea pig anaphylaxis test. The results using 7.5% icodextrin solution for sensitization displayed no response from the guinea pigs, while the horse serum treated positive controls displayed a strong positive response. There was swelling and edema in the paws due to this treatment, consistent with the type of allergenic response anticipated to be evoked by icodextrin.

Key findings:

Study no: 10652
Study type (if not reflected in title):
Volume #, and page #: vol. 11, pp. 1-16
Conducting laboratory and location: Baxter, Round Lake, Illinois
Date of study initiation: 4/21/2000
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: 7.5% Icodextrin in PD2 electrolyte solution, lot # DAX2035-3
Formulation/vehicle:

Methods:

TITLE

Evaluation of Potential for Icodextrin to Induce Anaphylaxis in Guinea Pigs

PURPOSE

To assess the potential of 7.5% Icodextrin in PD2 electrolytes to induce anaphylaxis in guinea pigs.

BACKGROUND

The Renal Division has developed a peritoneal dialysis (PD) solution specifically designed for the long dwell (8-16 hours). Icodextrin acts as a colloid osmotic agent to be used as a replacement for dextrose in PD solutions. Large molecular weight solutes, such as Icodextrin, offer an alternate class of osmotic agents to both affect dialysis (clearance and ultrafiltration) and resist carbohydrate absorption by the patient. The use of Icodextrin for the long dwell exchange may improve peritoneal dialysis therapy overall due to its isoosmolarity and relatively low glucose load. Because of the potential clinical therapy benefits in an end stage renal failure patient, slight to moderate immunologic responses may be acceptable and are clinically manageable. However, an anaphylactic episode would be unacceptable during therapy. Therefore, a study designed to assess 7.5% Icodextrin in PD2 electrolytes for anaphylaxis was necessary.

REGULATORY COMPLIANCE

The study was conducted in accordance with the FDA Regulations for Good Laboratory Practice, 21 CFR Part 58 (Ref 1) and the USDA Regulations for Animal Welfare, 9 CFR Parts 1, 2, and 3 (Ref 2).

TEST AND CONTROL ARTICLES

Test Article: 7.5% Icodextrin in PD2 electrolytes (sodium chloride, calcium chloride, magnesium chloride, and sodium lactate) supplied by the Renal Division, Baxter Healthcare Corporation.
- Lot Number: DAX2035-3
- Storage: Room Temperature

Positive Control Article: Serum Equine (horse serum), manufactured by ———
- Lot Number: 9783A
- CAT Number: 191355
- Expiration Date: Apr 11, 2003

Characterization and Stability: Characterization and stability testing of the test article is being conducted by the IV Systems Division, Baxter Healthcare Corporation for the Renal Division and can be traced through the lot number. The positive control article was a marketed product and was characterized by its labeling.

TEST SYSTEM

Species: Albino Guinea Pig

Stock: Hartley

Justification: The albino guinea pig was selected as the test system based upon established knowledge of its acceptability for use in anaphylaxis studies.

Source: Guinea pigs were obtained from ——

Body Weight Range: 301 - 335 g on study day 0.

Age: 4 weeks on study day 0

Number and Sex: 12 male guinea pigs (n=3/group).

Quarantine: 7 days

Identification: Guinea pigs were assigned a Baxter identification number upon receipt and were individually identified by an ear tag.

Health Status: No signs of clinical illness were observed in any of the guinea pigs. The guinea pigs were from a colony that was certified by the vendor to be free of specific pathogens as indicated upon receipt.

ANIMAL HUSBANDRY

Environmental Controls: Humidity greater than 70% relative humidity (RH) [70-74%RH] was observed for less than 6 hours on one day in the animal room due to high external environmental humidity. The high humidity condition was for a relatively short period and did not appear to affect the animals.

Caging: Guinea pigs were individually housed in suspended stainless steel cages.

Feed: Guinea pigs were provided certified guinea pig feed —— —— *ad libitum.*

Water: Distilled drinking water was provided *ad libitum.*

Feed/water Contaminants: There were no known contaminants in the feed or water that would interfere with this study.

EXPERIMENTAL DESIGN

The experimental design is shown in Table 1. Each guinea pig was administered a series of three intraperitoneal (IP) injections of either the test article or positive control article during the induction phase. Fifteen or 22 days after the initial IP injection, guinea pigs were intravenously challenged with the previously administered test article or positive control article via the marginal ear vein. Guinea pigs were continuously observed for signs of anaphylaxis for up to 15 minutes after challenge. Additional observations were performed at 20 and 30 minutes and 24 hours after challenge.

Table 1.
Experimental Design

Group	Article	Intraperitoneal Induction		Intravenous Challenge		No. of Animals
		Dosage	Days	Dosage	Day	and Sex
1	Icodextrin	0.5 ml	0, 2, 4	0.2 ml	15	3M
2	Icodextrin	0.5 ml	0, 2, 4	0.2 ml	22	3M
3	Horse Serum	0.1 ml	0, 2, 4	0.2 ml	15	3M
4	Horse Serum	0.1 ml	0, 2, 4	0.2 ml	22	3M

ASSIGNMENT TO STUDY

Twelve guinea pigs were randomly assigned three to a group for a total of 4 groups. The assignment scheme was provided by [redacted].

LABORATORY METHODS

Intraperitoneal Induction Phase: The intraperitoneal route of administration was selected for this study because it duplicates the route of patient exposure to the test article. The abdomen of each guinea pig was shaved prior to the initial dose (day 0). On day 0, 2 and 4, guinea pigs were weighed and 0.5 ml of Icodextrin (Groups 1 and 2), or 0.1 ml of horse serum (Groups 3 and 4) was administered intraperitoneally.

Intravenous Challenge Phase: On day 15 or 22, animals in groups 1 and 2, respectively, were administered 0.2 ml of icodextrin via an intravenous injection in the marginal ear vein. On day 15 or 22, animals in group 3 and two of the three guinea pigs in group 4, respectively, were administered 0.2 ml horse

serum via an intravenous injection in the marginal ear vein. Due to injection difficulties, one group 4 guinea pig received only 0.1 ml of horse serum. This guinea pig was exhibiting severe anaphylactic observations during the injection; therefore, the challenge dose was ended.

Observations:

Induction: Animals were observed immediately and approximately 30 minutes after each induction injection, then daily until the day of challenge.

Challenge: Animals were observed for signs of anaphylactic shock (e.g., hypoactivity, coughing or retching, cyanosis, dyspnea, lacrimation, convulsions, prostration, and/or death) continuously for up to 15 minutes post injection. Observations were recorded immediately and approximately 10, 20, and 30 minutes and 24 hours after challenge for the test article animals and immediately and up to 28 minutes after challenge for the positive control article animals. Guinea pigs challenged with horse serum on day 15 were euthanized within 3 minutes of dosing compared to 2 of the 3 guinea pigs challenged on day 22 euthanized greater than 15 minutes after dosing

Body Weight: Body weights (unfasted) were recorded prior to each induction treatment, and on day 7, 15, and 22.

Euthanasia: Positive control animals clearly exhibited severe clinical signs of anaphylaxis and were euthanized with an intracardiac injection of a sodium pentobarbital containing solution. All test article animals were euthanized by CO_2-induced hypoxia after the completion of the 24 hour post challenge observation period.

Necropsy: Necropsies were not performed.

Results:

the front right paw; however, the discoloration was not considered related to the challenge. Severe anaphylactic observations of retching, lacrimation, cyanosis, dyspnea, convulsions, prostration, and ataxia were seen in the positive control guinea pigs challenged with horse serum on day 15 and 22. The onset of severe anaphylactic observations was faster on challenge day 15 compared to challenge day 22.

Body Weight: Individual animal body weights are presented in Table 4. All animals gained weight throughout the study.

CONCLUSION

Under the conditions of this study, 7.5% Icodextrin in PD2 electrolytes did not induce anaphylaxis in the guinea pig after three intraperitoneal induction injections and an intravenous challenge on day 15 or 22. Positive control guinea pigs treated with horse serum in a similar manner did exhibit severe signs of anaphylaxis following intravenous challenge with horse serum.

Summary of individual study findings:

Conclusions:
Summary of individual study findings:
Study validity:
Study outcome:

Study title: Study 8L199: Antigenicity study of icodextrin.

Summary: This study was almost identical to study 10652, and had essentially identical results. No signs of anaphylaxis occurred in the guinea pigs in the icodextrin group, while the positive control group receiving horse serum all exhibited strong anaphylaxis (all died).

Key study findings:

Study no: 8L199
Volume #, and page #: vol. 11, pp. 17-35
Conducting laboratory and location:

Date of study initiation: March 19, 1998
GLP compliance: Yes
QA reports: yes ( ) no (X):
Drug, lot #, radiolabel, and % purity: Icodextrin, lot # DX-163J, purity 96.8%
Formulation/vehicle: PD2

Methods:

Results:

Outline of the Study

1. Title: Antigenicity Study of Icodextrin (Study No. 8L199)

2. Purpose: To assess the antigenicity potential of Icodextrin in guinea pigs

3. GLP: Japanese GLP Standards for Non-clinical Safety Studies on Drugs
(Ministry of Health and Welfare Ordinance No. 21, 1997)

4. Sponsor: Baxter Limited
4, Rokubancho, Chiyoda-ku, Tokyo, Japan
Responsible person: Toshio Hattori

5. Organization under contract :

6. Testing facility:

7. Study contributors:

Study director: _____ (sealed) Date: May 29, 1998

Other contributors: _____

8L199

8. Study date:	Initiation of the study	March	19, 1998
	Receipt of animals	March	25, 1998
	First sensitization treatment	March	30, 1998
	First challenge	April	13, 1998
	Second challenge	April	20, 1998
	Final observation	April	21, 1998
	Issuance of the final report	May	29, 1998

9. Unforeseeable circumstances that may have ill effects on the reliability of the study and deviation from the protocol:

9.1 Unforeseeable circumstances that may have ill effects on the reliability of the study:

None

9.2 Deviation from the protocol:

The cadmium content in the diet used in this study (— ppm) was higher than the specification in our laboratory (— ppm). However, this deviation from the protocol did not affect this study, because this deviation was very slight and it is reported that animals are not affected by the diet containing less than 0.5 ppm of cadmium (Exp. Anim., 1983, vol. 32, no. 1, 63-65).

10. Retention: The protocol, raw data, a sample of the test substance, documents and the final report will be retained in the archive of the ——— The sponsor and the testing facility will discuss regarding further retention of the raw data ten years after submission of the final report.

Table 1 Anaphylactic symptoms after challenge

Group	Sensitization antigen	Challenge antigen	Challenge	Number of animals	Anaphylactic symptoms[1)]				
					N	Sl	M	Se	D
Test substance	Test substance solution	Test substance solution	Day 15	3	3	0	0	0	0
			Day 22	3	3	0	0	0	0
Control for test substance	Electrolyte	Test substance solution	Day 15	3	3	0	0	0	0
			Day 22	3	3	0	0	0	0
Positive control	Horse Serum	Horse Serum	Day 15	3	0	0	0	0	3
			Day 22	3	0	0	0	0	3

[1)] N: Negative, Sl: Slight, M: Moderate, Se: Severe, D: Death

Summary of individual study findings:

Conclusions:

Summary of individual study findings:
Study validity:
Study outcome:

Study title: Study 97-I-689: Effects of icodextrin on chemotaxis of human leukocytes

Summary: This simple chemotaxis test looked at peripheral blood leukocytes from healthy human volunteers and looked at response to saline, 7.5, 15 and 25% icodextrin, electrolyte solution, and a positive control of zymosan-treated serum. Results showed no chemotactic response to the icodextrin solutions, only to the positive control. However, acidic environments inhibit leukocyte chemotaxis.

Insert info

Key findings:

Study no: 97-I-689
Volume #, and page #: vol. 11, pp. 36-48
Conducting laboratory and location:
Date of study initiation: March 30, 1998
GLP compliance: No
QA reports: yes ( ) no (X):
Drug, lot #, radiolabel, and % purity:

Materials

1. Test material and reference materials

1) Test material

Icodextrin:

Chemical name: α 1, 4, polyglucopyranose

Molecular formulation: $C_6H_{12}O_6[C_6H_{10}O_5]_nC_6H_{12}O_6$

Appearance: Amorphous white powder

7.5% Icodextrin electrolyte solution (Lot No. BLID-98-03)

15% Icodextrin electrolyte solution (Lot No. BLID-98-02)

25% Icodextrin electrolyte solution (Lot No. BLID-98-01)

2) Reference materials

Electrolyte solution (Lot No. BLID-98-04)

Ingredient (in 100 mL):

NaCl	538	mg
$CaCl_2 \cdot 2H_2O$	25.7	mg
$MgCl_2 \cdot 6H_2O$	5.08	mg
Sodium lactate	448	mg

Dianeal PD-2 2.5 (Lot No. PP2505)

Ingredient (w/v%):

Glucose	2.27
NaCl	0.538
$CaCl_2 \cdot 2H_2O$	0.0257
$MgCl_2 \cdot 6H_2O$	0.00508
Sodium lactate	0.448

These substances were supplied from the sponsor on April 10, 1998.

Storage of test substances: Room temperature

Methods

Polymorphonuclear leukocyte

Venous blood in an amount of 20~30 mL/person was obtained from 5 healthy adult men using heparin as an anticoagulant. Six percent dextran in saline solution in a volume of 1/4 of the blood was added and mixed well, and the resultant solution was left standing at room temperature for 30 minutes. The plasma layer was layered on Ficoll solution and then centrifuged at 500×g for 15 minutes. The precipitated polymorphonuclear leukocytes were prepared into a suspension of about 5×10^7/mL in Hanks solution.

Inactivated human sera

The sera obtained from 5 subjects were mixed and processed at 56°C for 30 minutes.

Sera treated with zymosan

Zymosan (0.025 g) was washed with phosphate buffer. To this, 1 mL of serum (obtained by mixing the sera from more than 5 subjects) was added and incubated for 1 hour at 37°C. The supernatant obtained after centrifugation with 500×g for 10 minutes was incubated for 30 minutes at 56°C.

Agarose plate

Ten mL of sterilized distilled water was added to 0.24 g of agarose and this was boiled in a bath for 15 minutes. The resultant solution was further dipped in a bath for about 5 minutes at 47°C. Two mL of 10-fold Hanks solution, 2 mL of the inactivated human serum, 0.6 mL of 1M HEPES buffer and 5.4 mL of distilled water for injection were mixed and warmed for about 5 minutes in a 47°C bath. The resultant solution was mixed well with the previously prepared agarose solution, and 5 mL of this was dispensed in each petri dish, which was hardened in a refrigerator. Three plates were prepared for each case.

Chemotaxis test

Using a punch, 8 rows of dents each measuring 3 mm in diameter were prepared radially with an interval of 3 mm (7 of the 8 rows were used) in an agarose plate. Using a micropipet, polymorphonuclear leukocyte suspension (about $5\times10^5/10\ \mu L$) was filled in the central dent of each row, the same amount of saline solution, electrolyte solution, the zymosan-treated serum, 7.5%, 15%, 25% Icodextrin solutions and Dianeal PD-2 2.5 in the outer dents, and Hanks solution in the inner dents. All the 3 plates were processed the same. After incubation at 37°C for 2 hours, the agarose plate was fixed with methanol and formalin. After washing with methanol, Wright's staining was done. The distance of taxis (the distance of outward movement of leukocyte, chemotaxis) and random movement (the distance of inward movement, spontaneous migration) were determined in triplicate, and the assessment was made by using chemotaxis/spontaneous migration as the chemotactic index.

Results:

Results and discussion

Saline solution, electrolyte solution, 7.5%, 15% and 25% Icodextrin solutions and Dianeal PD-2 2.5 did not influence the chemotaxis of human leukocyts. On the other hand, chemotaxis was observed with the zymosan-treated serum which was used as the positive reference (Table).

The present results clearly indicated that the decrease in leukocytes by the administration of Icodextrin is not caused by the chemotaxis of leukocytes.

Summary of individual study findings:

Conclusions:

Summary of individual study findings:
Study validity:
Study outcome:

Immuno-toxicology **summary**: Anaphylactic Studies: Warnings have been issued on the ability of icodextrin solutions to induce skin reactions reflective of a developing allergenic response in human patients on icodextrin therapy (refs). This has led to discontinuation of this therapy for several patients. This is probably related to the allergic responses sometimes seen in response to various dextrans (refs). Most of the dextran studies have utilized a rat model system rather than the typical anaphylactic guinea pig model system (). The studies used for anaphylaxis reported by the sponsor only used the guinea pig model, with negative results. No reports of comparison with the rat paw edema model system is provided, a system frequently used for examining dextran allergies. This would be informative since work in the rat system also models a hapten therapy that blocks dextran allergies in humans. This could be useful for maintaining patients on icodextrin therapy.

Immuno-toxicology conclusions:

Labeling recommendations: Icodextrin is known to induce allergic responses in humans and this may lead to discontinuation of therapy.

ADDENDUM TO REVIEW: ESTELLA BARRY'S REVIEW OF IND: —
(if necessary)

APPENDIX/ATTACHMENTS:
Study title:

Key study findings:

Study no:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA report: yes ( ) no ( )
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle:

Methods (unique aspects):
Dosing:
Species/strain:
#/sex/group or time point (main study):
Satellite groups used for toxicokinetics or recovery:
Age:
Weight:
Doses in administered units:

Route, form, volume, and infusion rate:

Observations and times:

Clinical signs:
Body weights:
Food consumption:
Ophthalmoscopy:
EKG:
Hematology:
Clinical chemistry:
Urinalysis:
Gross pathology:
Organs weighed:
Histopathology:
Toxicokinetics:
Other:

Results:

Mortality:
Clinical signs:
Body weights:
Food consumption:
Ophthalmoscopy:
Electrocardiography:
Hematology:
Clinical chemistry:
Urinalysis:
Organ weights:
Gross pathology:
Histopathology:
Toxicokinetics:

Summary of individual study findings:

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

James Willard
8/13/01 01:48:58 PM
PHARMACOLOGIST

Albert Defelice
8/15/01 05:27:44 PM
PHARMACOLOGIST

D7

MAY 2 9 1998

interoffice
MEMORANDUM

ADP

to: To The File through AF DeFelice, Ph.D (Team Leader)
IND [redacted] (Serial No. 001; 09-15-97) Icodextrin 7.5% Peritoneal Dialysis Sol.

from: Estela A. Gonzalez Barry, M.S. EB

subject: Request for Reviewers Comments on IND Preclinical Pharmacology/Toxicology Studies.

date: May 28, 1998

Baxter Healthcare has no plans to conduct additional animal studies in support of the safety of Icodextrin 7.5% Peritoneal Dialysis Solution (Extraneal) and is interested in knowing whether FDA considers that the previously submitted nonclinical data with the solution to be sufficient.

In the Original IND Submission (Serial No. 000), the drug sponsor submitted what PHARMACOLOGY considers at this time sufficient nonclinical studies to characterize the safety of the solution for its intended use- for removing certain substances from the body in patients with end-stage renal disease.

Briefly, marketed peritoneal dialysis solutions contain physiologic amounts of sodium, calcium, magnesium, glucose and a buffer. Glucose at specified concentrations provides an osmolar gradient that permits ultrafiltration of fluid. A small volume of the peritoneal fluid is absorbed through the diaphragmatic lymphatics, limiting net ultrafiltration. Peritonitis is the most common serious complication of peritoneal dialysis*.

In the proposed peritoneal dialysis solution, icodextrin is used instead of glucose. Icodextrin is a large molecular weight soluble glucose polymer which is isolated by fractionation of maltodextrin. Nonclinical studies performed to characterize the safety of icodextrin included, in addition to pharmacology and pharmacokinetics studies, acute toxicity studies (by iv/ip routes in mouse and rat), repeat dose studies (ip in rats; ip/iv in dogs up to 28 days with recovery periods), and for genotoxicity (Ames test, chromosomal aberration test in CHO, and micronucleus test).

Available label for another Baxter peritoneal dialysis solution revealed that no additional toxicology studies were conducted to get approval for Dianeal. Telecon with S.F. Hoff (Baxter Renal Div.) revealed that no additional nonclinical studies have been required of them in the past and no additional studies have been conducted. Communications with R. Steigerwalt, Ph.D., Team Leader in HFD-510 (Division that usually reviews these solutions) corroborated all of these statements. (See attached Telefax dated 05-22-98). Based on this information. PHARMACOLOGY considers that at this time, no additional nonclinical studies are required.

* Recomendations for treatment provided by Int. Soc. for Periotoneal Dialysis:
http://www.ispd.org
cc:GBuehler, RHPM

TELEFAX

TO: Estella Barry

FAX: 594-5495
PHONE: 594-5300

FROM: Ron Steigerwalt
Food and Drug Administration
Division of Metabolic and Endocrine Drug Products
5600 Fishers Lane HFD-510
Rockville, MD 20857-1706

FAX: (301) 827-0878
PHONE: (301) 827-6369

RE: Labeling for LVP's
DATE: May 22, 1998
PAGES: 2 (inclusive)

Estella,
I've sent a copy of the page of an lvp that contains the labeling we usually request when the studies for pharm/tox sections have not been done. Under nursing mothers, we often use a slightly different statement as follows:

"It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised [drug name] is administered to a nursing mother."

Either option for the nursing mothers should be fine.

/S/
