[redacted] was not genotoxic[104] in the following [redacted] tests, Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the [redacted] human subcutaneous dose, based on body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed[105].

[redacted] "Pregnancy- Teratogenic Effects-Pregnancy Category [redacted]

[redacted]

[redacted]

[

]

Nursing- It is unknown whether ——— is excreted in ——— human milk. For this reason, caution should be exercised when ——— is administered to a nursing mother.[109] ———

Justification for change:

1. Slightly, but not statistically significant increases (p=0.062) in mammary gland tumors were observed between X14 and regular human insulin (at 32-times the human dose) in a 52-week toxicity study in rats (QA certified study). X14 also had a higher potential in promoting benign and combined (benign + malignant) mammary gland tumors compared to vehicle controls (p=0.003-0.0039), than human insulin compared to vehicle controls (p=0.24). However, X14 is not genotoxic, and slight increases in the tumorigenic potential of X14 compared to human insulin is observed at 32-times the maximum recommended starting human dose. Therefore, under 'Carcinogenicity', the reviewer is suggesting the above text for labeling.

[

]

3. Studies in rats were conducted up to 32-times the human dose, and in rabbits up to 3-times the human dose respectively, under ——— ', the reviewer is suggesting a change in title and the text for labeling.

Recommendation:
From the preclinical standpoint, approval of this application is recommended, pending acceptable labeling modifications are made.

/S/

Indra Antonipillai, Ph.D.
Pharmacologist, HFD-510

cc: NDA Arch
HFD510
HFD510/antonipillai/steigerwalt/koller/jrhee
Filename:NDA20-986

Concur: /S/
7/22/99
See Team Leader Memo to file

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Appendix:
Histopathology Inventory for NDA 20-986, 1, &12 month studies in rats & 1, 3 and 12 month studies in dogs

Species	Rat				Dog			
	1-month	12-mont			1-month	3-month	12-mont	
Adrenals		X*			X*	x*	x*	
Alimentary tract		X*			X		x	
Aorta		X			X	x	x	
Bone Marrow smear								
Bone (femur)								
Brain		X*			X*	x*	x*	
Cecum						x	x	
Cervix								
Colon						x	x	
Duodenum						x	x	
Epididymis						x*		
Epididymides		x*						
Esophagus							x	
Eyes		X			X	x	x	
Fallopian tube								
Femur		X			X	x	x	
Gall bladder					x	x	x	
Harderian gland		x						
Head		x						
Heart		X*			X*	x*	x*	
Hyphophysis								
Ileum						x	x	
Injection site		X			X		x	
Jejunum						x	x	
Kidneys		X*			X*	x*	x*	
Lachrymal gland							x	
Larynx and pharynx		X			X			
Liver		X*			X*	x*	x*	
Lungs		X*			X*	x*	x*	
Lymph nodes, cervical		X			X	x	x	
Lymph nodes, mesenteric					x		x	
Mammary Gland		X			x	x	x	
Ovaries		X			X	x*	x*	
Pancreas		X			x	x	x*	
Parathyroid								
Peripheral nerve								
Pharynx								
Pituitary		X			X*	x*	xx*	
Prostate		X			x*	x*	x*	
Rectum						x		
Salivary gland		X*			x*	x	x*	
Sciatic nerve		X			X	x	x	
Seminal vesicles		x*						
Skeletal muscle		X			x	x	x	
Skin		x			X	x	x	
Spinal cord		X			X	x	x	
Spleen		X*			x*	x*	x*	
Sternum		X			X	x	x	
Stomach						x	x	
Testes		X*			X*	x*	x*	
Thymus		X*			X*	x*	x*	
Thyroid		X*			X*	x*	x*	
Tongue		X			x		x	
Trachea		X			x	x	x	
Urinary bladder		X			X	x	x	
Uterus		X*			X*	x*	x*	

Vagina		X			X	x	x	
Zymbal gland								

- organ weight obtained

APPEARS THIS WAY
ON ORIGINAL

LIV-File

OCT -2 1995

IND# ———— October 2, 1995

Sponsor: Novo Nordisk Pharmaceuticals Inc., Princeton NJ
Contact: Markus F. Herzig Tel(609)987-5800

Submission Date: 06/29/1995

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW

1. Drug: Insulin X14 (B28 Asp-insulin)

2. Chemistry: Recombinant human insulin, of which proline at position B28 was substitued with aspatic acid.

3. Pharmacological class: Insulin analogue

4. Indication: Type I diabetes

TABLE OF CONTENTS

/S/
Herman M. Rhee, Ph. D.

cc: Original IND, HFD-510
A. Jordan/H. Rhee

/S/
10/2

IND# ———— October 2, 1995

Sponsor: Novo Nordisk Pharmaceuticals Inc., Princeton NJ
Contac: Markus F. Herzig Tel(609)987-5800

Submission Date: 06/29/1995

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW

1. Drug: Insulin X14 (B28 Asp-insulin)

2. Chemistry: Recombinant human insulin, of which proline at position B28 was substituted with aspartic acid.

3. Pharmacological class: Insulin analogue

4. Indication: Type I diabetes

5. Clinical:

The objective of this trial is to compare the effects of three different injection sites (deltoid, thigh and abdomen) on the action profile of Insulin analogue X14 by means of a euglycemic clamp technique. There will be six study days separated by at least a week, and on each of these days the subjects will receive a single dose (0.2 U/kg) of Insulin X14 or Novolin-R.

6. Previous human study:

Seventy-two patients and volunteers from Germany, United Kingdom and Netherlands used insulin X14. The doses were from 0.05 U/kg to 0.1 U/kg. None of the subjects dropped out of any of the studies due to reported adverse events. There were no serious adverse events reported in any of the clinical trials. The majority of non-serious adverse events were hypoglycemia, usually mild, and generally less with Insulin X14 when compared with Soluble Human Insulin.

A. PHARMACOLOGIC STUDIES

1. Receptor Binding Studies

Insulin x14 and four other analogues were studied using intact human hepatoma cells(Hep G2) by a assay. Results are listed below:

Substance	Relative insulin receptor affinity (% of Rh-insulin)	Relative IGF-I receptor affinity (% of IGF-I)
Human Insulin	100	0.08
IGF-I	3.2	100
Insulin X14	80	0.06
Insulin X10	327	0.32

2. In vivo Effect on Glucose Metabolism

At least three New Zealand White rabbits, which had been fasted overnight, were used. Insulin(batch 11517 or 891100) or Insulin X14(Batch P10191) were injected intravenously via the central vein at doses of 50, 100 and 150 mIU. Insulin X14 produced similar hypoglycemic effect to Actrapid in terms of onset of action and duration of action.

3. Mitogenicity

a) Method: A number of in vitro mitogenicity assays have been performed to compare the effect of human insulin and insulin analogues such as Insulin X14 in typical cell culture systems. The cultured cells were rat aortic smooth muscle cells, mouse NIH 3T3 fibroblasts, and Chinese Hamster ovary cells. The mitogenicity was measured by the ability to stimulate ^{3}H-Thymidine incorporation into DNA.

b) Results:

Substance	No.of Assays	Potency Ratio to H. Insulin	Std.-Error
Human Insulin	-	1.00	-
Insulin X14	6	0.94	±0.11

c) Conclusion: Mitogenic potencies of Insulin X14 and human insulin are similar.

4. Cardiovascular effects of Insulin X14 in the anesthetized pig(study No. 15887)

Three female SPF cross-breed(Dansk Landrace/Yorkshire) pigs, 22-25 kg body weight, were anesthetized with pentobarbital. Insulin X14 (0.09 or 0.9 IU/kg) was given intravenously. Systematic BP, pulmonary BP, central venous BP, cardiac output and heart rate were determined as a function of drug dose and exposure time to the drug. Insulin X14 did not have acute effects on the cardiovascular system. But, the systemic BP decreased throughout the studies performed independent of drug treatment time. The decrease of blood pressure might relate to the hypoglycemic action of the peptide.

5. Summary and conclusion

Insulin X14 showed a low affinity for the IGF-I receptor as human insulin did. Activation of the insulin receptor kinase(data not shown) was proportional to receptor affinity, indicating that binding to the receptors of Hep G2 cells is relevant. Insulin X14 analogue shows effects equal to human insulin on total glucose utilization in vivo when given in equimolar amounts. Insulin X14 appeared to be effective in lowering blood glucose without an acute effect on systemic blood pressure.

B. PHARMACOKINETICS

A traditional ADME-package has not been performed on Insulin X14 because of the lack of a specific immunoassay for Insulin X14. However, the sponsor was able to measure the plasma profile of Insulin X14 by means of

[redacted] method using Insulin X14 as standard in pigs. Five female normal pigs (cross-bred from Danish Landrace and Yorkshire) were fasted overnight. Insulin X14 was given either intravenously (0.15 nmol/kg) or subcutaneously (0.6 and 1.2 nmol/kg) to the pigs for 7 days. Time-depended analysis of blood samples of Insulin x14 indicated that Insulin X14 was absorbed faster(faster onset and shorter duration) than soluble human insulin(Actrapid). Reduction in plasma glucose in the pigs was dependant on Insulin X14 dose. A [redacted] study of rats receiving I.V. bolus injections of radiolabelled human insulin and Insulin X14 showed no significant differences between human insulin and Insulin X14.

C. TOXICOLOGY

1. Acute Toxicity

1-1. Insulin x14: Acute Subcutaneous toxicity study in the mouse — Report #87/NLP053/913) and in the rat — Report #87/NLP052/912)

a. Methods: Five Albino mice(CD-1) or CD rats/sex/group were administered a single subcutaneous injection of Insulin X14 at doses of 0(control), 62.5, 250, 1000 or 4000 IU/kg. Three separate inspections were made during the first hour after dosing and two further inspections during the remainder of Day 1. The type, time of onset and duration of reactions to treatment were recorded.

b. Results: There were no deaths. No systemic sign of reaction to treatment was noted during the two-week observation period. This drug had no effect on body weight in both the mice and the rats.

2. Subacute/Chronic Toxicity

1-2. Insulin x14: Toxicity Study by Subcutaneous Administration to CD Rats for 4 Weeks ——→Report #88/NLP059/419).

a. Methods: Forty-eight SD rats(CD)/sex/group were administered Insulin X14 at doses of 0, 12.5, 50 and 200 IU/kg/day for a month. In this study the sponsor used 5 different batches(10287, 10387, 10487, 10587 and 10687) of Insulin X14.

b. Results: Dominant clinical signs were changes at the injection sites, which were reddening, swelling, weeping, ulceration, encrustation or bleeding. There was no mortality except a female which was killed accidently during blood sampling. Insulin X14 had no effects on food and water consumption, and bodyweight. There were no inter-group differences in hematologic parameters, organ weight and urine analysis. Blood chemistry was not altered by the drug except aspartate amino-transferase activity was significantly elevated($p<0.01$) in both sexes. There were no histopathologic changes in any tissues which were ascribed to the treatment with the drug, although the incidence of periacinar hepatocyte vacuolation was higher in male rats that were treated with Insulin X14(200 iU/kg/day).

1-3. Three Month Study in Rats(Study #SX12650)

a. Method: Fifteen Mol:Wist rats/sex/groups were given Insulin X14 at doses of 0, 12.5, 50 and 200 iU/kg/day for 3 months.

b. Results: Twelve rats died during the treatment period, primarily in the high dose group. The cause of death is believed to be in all cases due to an insulin shock. There

was a treatment related increase in body weight and body weight gain in the high dose females. No differences in water and/or food consumption was observed between test groups and controls. Serum alanine aminotransferase was dose-dependently low in mid and high dose groups. Serum protein levels were dose-dependently low in females of all dose groups. A number of effects of the drug on absolute and relative organ weight were noted, but most were believed to be incidental. For instance, high absolute and relative thymus weight in the high dose group were thought to be due to increased fat deposition. The only gross pathology and microscopic findings were changes at the injection site, which were caused by the ——— m-cresol.

c. Conclusion: Insulin X14 administered subcutaneously daily for 3 months caused no adverse effects to rats at all three dose levels. Deaths were probably due to an exaggerated pharmacodynamic reaction.

1-4. Insulin X14 Exploratory 12 Months Toxicity Study in Rats — Study #930803)

The purpose of this exploratory study was to investigate the effect on the incidence of mammary tumors in female CD rats treated with 200 U/kg/day (200-fold the expected clinical dose) of Insulin X14 treatment for a year. The sponsor did this study because ——— produced mammary tumors in female rats under similar experimental conditions.

a. Methods: Twenty female SPF Sprague-Dawley rats (CD)/group were administered subcutaneously 0 (control) or 200 U/kg/day of ——— Insulin X14 or Actrapid for 12 months.

b. Results:

1) Mortality: All rats found dead in their cages had empty stomachs, suggesting that severe hypoglycemia was the cause of death. Mortality data are summarized below.

Treatment	[illegible]	X14	Actrapid	Control
Animal #	20	20	20	20
Deaths #	5	3	8	0
Sacrificed Animal #	1	2	0	1
Survivors#	14	15	12	19

2) Clinical signs: No general signs were noted even though visually detectable tumors were observed as indicated under item 4) Pathology and Histopathology Section.

3) Body Weights: The mean observed final weight for both Insulin X14 and Actrapid was 6% higher than for untreated, but the difference was not statistically significant.

4) Pathology and Histopathology: In all groups of rats including the control, tumors in mammary glands were found to be of the same type. They were large subcutaneous masses with a diameter from a few mm up to 7 cm. The cut surfaces of these tumors showed a lobular arrangement with solid grey interstitia rich in collagen and protruding lobules of soft parenchyma varying in size and color.

By histological examination all animals in all groups had hyperplasia of mammary glandular epithelial cells. No significant differences in severity were found in the groups when compared to the Actrapid group. The majority of mammary gland tumors were benign and classified as fibroadenomas. The

malignant mammary gland tumors were classified as adenocarcinomas but with no metastasis to the axillary or inguinal superficial lymphatic nodes. Pathological findings on adenocarcinomas are summarized below.

Treatment	—	X14	Actrapid	Control
Examined#	17	18	17	20
Benign #	11	7	8	4
Malignant#	3	4	3	1

By comparison of each pair of groups in the combined analysis of incidental benign mammary tumors the only significant differences were between —— and untreated and between Actrapid and untreated as shown below.

Test 1	Test 2	Positive	Expected	Error	P
—	Actrapid	11	9.5	1.4	0.30
X14	Actrapid	7	8.3	1.6	0.39
Control	Actrapid	4	7.2	1.4	<0.05
—	Control	11	6.6	1.6	<0.01
X14	Control	7	4.9	1.3	0.16
—	X14	11	8.9	1.7	0.20

However, statistical analysis of malignant mammary tumors indicate that no differences were seen in the incidences of the tumors between any of the preparations tested as shown below.

Test 1	Test 2	Positive	Expected	Error	P
—	Actrapid	3	3.2	1.1	0.83
X14	Actrapid	4	4.1	1.2	0.92
Control	Actrapid	1	2.5	0.9	0.12
—	Control	3	1.7	1.0	0.18
X14	Control	4	2.8	1.0	0.20
—	X14	7	3.3	1.2	0.81

C. Summary and Conclusion:

Treatment of female CD rats with subcutaneous administration of 200 U/kg/day of human insulin (Actrapid) or ——— X14 and —— reduced survival due to presumable drug-induced hypoglycemia. A visual inspection of all animals indicates that the incidence of palpable masses in the group treated with ——— were significantly higher and the onset of palpable masses was faster when compared with the other three groups. Although the number of fatal adenomas was so small that they showed no significant differences, combined analysis of incidental benign adenomas in the mammary gland indicated that ——— was associated with high incidence of the tumor. In the analysis Actrapid itself was positive($p<0.05$) compared to control and none of the preparations was positive in combined analysis of malignant tumors. The study showed that no pituitary gland tumors were associated with the treatment of Actrapid or insulin analogue . In conclusion, this study demonstrated that the tumorigenic potential of Insulin X14 was no greater than endogenous insulin in the preliminary experiments.

1-4. Insulin X14: Maximum Tolerated Dose Study by Subcutaneous Administration to Beagle Dogs(Doc. Id. 95-0019-01)

a) Methods: Two male and two female beagle dogs were administered subcutaneously Insulin X14(Batch #10487) for 17 days as follows:

Day number	Dose(IU/kg/day)	Volume(ml/kg)
1 to 3	2.0	0.020
4 to 7	3.0	0.030
8 to 10	3.5	0.035
11 to 17	4.0	0.040

b) Results: There were no deaths and no clinical signs except a subcutaneous mass at the injection site. The drug had no effects on food and water consumption, bodyweights, hematologic parameters, and blood chemistry. Blood glucose levels were reduced by the treatment. Organ weights and macroscopic pathology were not altered by the administration of Insulin X14.

c) Summary and Conclusion: The administration of Insulin X14 at dosages of 2.0 to 4.0 IU/kg/day failed to elicit any evidence of toxicity in dogs. The administration caused hypoglycemia on Day 16, which suggests bioavailability of the test substance following subcutaneous administration. It appears that the maximum tolerated dose was at least 4.0 IU/kg/day in dogs.

APPEARS THIS WAY
ON ORIGINAL

1-5. Insulin X14: Three Months Subcutaneous Toxicity Study In the Dog(Doc ID.95-0013-01)

a) Methods: Four beagle dogs/sex/group were administered Insulin X14(batch P-10791 and P-10691) subcutaneously at doses of 0(medium), 1 or 4 IU/kg/day for 90 days.

b) Results: During the course of the dosing period there were a number of hypoglycemic episodes, particularly after the high dose, which were treated with glucose administration. Other clinical signs were vomiting, and soft feces with or without blood in all groups. There were no clear drug effects on ophthalmoscopy, hematology or blood chemistry. Macroscopic and microscopic examinations showed no treatment related changes.

c) Conclusion: Insulin X14 appears to be reasonably nontoxic since it did not produce unexpected adverse effects in dogs after its daily subcutaneous administration for 3 months.

D. IMMUNOTOXICITY

a) Method: Immunogenicity of mono component Insulin X14 was compared relatively to mono component human insulin, bovine insulin and [redacted] porcine insulin in rabbits and transgenic mice. The drugs(120 μmol) were injected to rabbits subcutaneously twice a week and serum insulin antibody binding was estimated by use of mono-^{125}I-Insulin. Similar experiments were also performed in transgenic mice which were transfected with human insulin gene.

b) Results/Conclusions: Insulin X14 was less immunogenic than porcine insulin or bovine insulin. But, it was more immunogenic ($P < 0.001$) than human insulin in rabbits. In the transgenic mice, neither Insulin X14 nor human insulin(rDNA)

elicited antibody formation, which suggests Insulin X14 has an immunogenic potential similar to human insulin.

E. REPRODUCTIVE TOXICITY

1. Study in the Pregnant Rat by subcutaneous Injection(Report No. — /99. — Study No. 930498)

a) Method: Ten pre-mated female SD rats/group were given Insulin X14 at doses of 0(vehicle), 5, 25 and 100 U/kg/bid from day 5 to day 16 post coitus. On day 20, females were sacrificed and subjected to post mortem examination.

b) Results: No mortalities or unusual clinical signs were observed. Both Insulin X14 and Actrapid at 100 U/kg/bid produced a similar degree of hypoglycemia, although the former increased early embryonic deaths slightly.

2. Effect on Pregnancy of the Rabbit — '100. — Study No. 940050)

a) Method: Six pre-mated female rabbits/group were given Insulin X14 subcutaneously at doses of 0, 6.25, 12.5 and 25 U/kg/bid from day 6 to day 18 post coitus. On day 29, females were sacrificed and subjected to postmortem examination.

b) Results: Insulin or Insulin X14 treatment resulted in increased weight gain and food intake, and lowered plasma glucose levels. The treatment was also associated with increased embryofetal death(abortions) at the highest dose(25 U/kg/bid).

F. MUTAGENIC TOXICITY

1. Reverse Gene Mutation in Bacteria

a) Method: Insulin X14 and human insulin were assayed for the ability to induce reverse mutations in bacteria using a treat and plate protocol as both test articles were known to contain some ———— Tests were performed using 4 Salmonella typhimurium and 2 E. Coli strains, both in the presence and absence of S-9. Treatments were performed up to 5000 µg/ml.

b)Results: Treatment with Insulin X14 caused occasionally a small increase in revertant numbers. But, it was not reproducible and might be due to chance.

2. Chromosomal Aberration in Human Lymphocytes in Vitro

a) Method: Insulin X14 was tested in an in vitro cytogenetics assay in 2 independent experiments. Treatments were carried out both in the presence and absence of S-9 metabolic activation and to the maximum concentration(5000 µg/ml). The same dose of human insulin was also used as a reference.

b) Results: Neither Insulin X14 nor human insulin were seen to induce chromosome aberrations in either experiment, in the presence or absence of S-9 metabolic activation.

3. Micronuclei in Mouse Bone Marrow Cells

a) Method: Mice were given Insulin X14 or human insulin subcutaneously on 2 occasions, 10 hours apart. The maximum dose was 1000 U/kg. Groups of male and female mice were sacrificed 24 and 48 hours after the second administration and micronuclei were scored in at least 2000 polychromatic erythrocytes(PCEs) per animal.

b) Results: Blood glucose levels fell immediately post treatment. Neither Insulin x14 nor human insulin caused any

change in PCE to NCE(Normochromatic erythrocyte) ratio. And there was no statistically significant increase in the frequency of micronucleated PCEs.

4. Unscheduled DNA Synthesis in Hepatocytes of Rats Treated in vivo

a) Methods: Male and female rats were dosed with Insulin X14 or human insulin at doses up to 1000 U/kg, using the I.V. route. Animals were dosed on 2 occasions, 10 hours apart, and sacrificed 2 hours following the second administration. The maximum cumulative dose was equivalent to 2000 U/kg. Hepatocytes were isolated from groups of 4 animals, prepared and assessed for unscheduled DNA synthesis.

b) Results: The treatments did not elicit any obvious toxic signs but blood glucose levels fell soon after dosing. Net grain counts(nucleus-cytoplasm) remained normal and the percentage of cells in repair was consistently low(<2% when > 20% is considered positive) across all dose groups. Thus, no evidence of unscheduled DNA synthesis induced by either insulin or Insulin X14 was seen.

G. COMMENTS AND CONCLUSIONS

The sponsor used many different batches of Insulin X14 for their pharmacologic and toxicologic studies. The reviewer assumed the Insulin X14 batches were chemically comparable. It appeared that Insulin X14 was effective in reducing blood glucose dose dependently. It's onset of action may be faster than the onset of a human insulin preparation(Actrapid) with a slightly reduced duration of action. The primary adverse effect of Insulin X14 was hypoglycemia and other drug-induced toxicities may be comparable to insulin. Since [redacted] produced tumors in SD rats,

careful evaluation of the drug's carcinogenic potential should be continued.

H. RECOMMENDATIONS(Letter to the sponsor)

1. In an earlier study with ——— in rats, 200 IU/kg/day of Actrapid injected daily for one year did not produce any mammary tumors. In the later study — Study #930803) using the same strain of rat, Actrapid produced 11/17 benign or malignant mammary tumors. Is there an explanation for the discrepancy in the results of the two studies.

2. Please submit the results of mitogenicity study with Insulin X14 in cultured rat aortic smooth muscle cells and mouse NIH 3T3 fibroblast cells(Study No. 940072).

/S/

Herman M. Rhee, Ph. D.

/S/

10/2

cc: Original IND, HFD-510
A. Jordan/H. Rhee

APPEARS THIS WAY
ON ORIGINAL

Div File

MAR 18 1996

IND# [redacted] March 6, 1996
Sponsor: Novo Nordisk Pharmaceuticals Inc., Princeton NJ
Contact: Lynn Joesten Tel(609)987-5800
Submission Date: 02/05/1996

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
AMENDMENT S#002 [redacted]

1. Drug: Insulin X14 (B28 Asp-insulin)
2. Chemistry: Recombinant human insulin, of which proline at position B28 was substitued with aspatic acid.
3. Pharmacological class: Insulin analogue
4. Indication: Type I diabetes

A. BACKGROUND

In this amendment the sponsor tried to respond to our Divisional questions raised on October 22, 1995. To resolve the discrepancy between their previous studies (#930803) for 1-year carcinogenicity study in SD rats with human insulin(Actrapid), the sponsor initiated another 52-week study in CD Sprague Dawley rats at [redacted] #940267).

B. STUDY DESIGN

[redacted] Crl:CDBR Sprague Dawley rats (20 rats/sex/group) were administered human insulin subcutaneously at doses of 60 and 150 IU/kg/day for a year.

C. RESULTS

A dose related increase in weight gain and mortality when compared to saline control was shown. A significant increase in the incidence of mammary tumors, both benign and malignant combined, and malignant alone, in female rats in high dose group as summarized below.

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Appendix 2.2

TABLE 3.
HISTORICAL CONTROL DATA FOR MAMMARY FIBROADENOMAS, ADENOMA AND ADENOCARCINOMA FOR FEMALE [redacted] CD SPRAGUE DAWLEY RATS FROM TWELVE 52 WEEK TOXICITY STUDIES.

Study Code	A	B	C	D	E	F	G	H	I	J	K	L
Rats having tissue examined by microscopy	20	22	20	20	24	15	20	20	20	15	20	20
Rats bearing mammary tumours	0	3	0	0	1	2	2	0	1	1	5	1
Rats with fibroadenomas	0	2	0	0	0	0	0	0	0	0	5	1
Rats with adenomas	0	1	0	0	0	0	0	0	1	0	0	0
Rats with adenocarcinomas	0	1	0	0	1	2	2	0	0	1	0	0
Rats with >1 mammary tumour	0	1	0	0	0	0	0	0	0	0	1	0
Total number of mammary tumours in group	0	4	0	0	1	2	2	0	1	1	6	1

Historical data from [redacted] (collected over the last few years).

APPENDIX 2.1

TABLE 2.
HISTORICAL CONTROL DATA ON MAMMARY TUMOURS IN FEMALE CD SPRAGUE DAWLEY AND FISHER 344 RATS FROM CARCINOGENICITY STUDIES.

Laboratory	A	B	
Strain of rats	Fisher 344	Fisher 344	S. Dawley
Duration	102-107w	104-106w	98-128w
Number of rats necropsied	459	450	1204
Number of groups	23	9	23
MAMMARY GLAND TUMOURS % INCIDENCE			
Range - adenomas	0-6	14-38	27-72
Adenocarcinomas	0-5	0-4	6-40
Average adenomas	13	24	49
Adenocarcinomas	1	2	20
Combined average	14	26	69

Historical data from:
"Spontaneous tumours in control F344 and ——— CD rats and ——— —— CD-1 and B6C3HF1 mice"
Sanford S.P. et al., Toxicology Letters, 11, p103-110, 1982

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ON ORIGINAL

INCIDENCE OF MAMMARY TUMOR IN FEMALE RATS AFTER HUMAN INSULIN

Dosage(IU/kg)	Cont*	60	150
Animals examined by microscopy	20	20	20
Animals examined at termination	20	15	10
Animals bearing mammary tumors	6	8	11
Animals with fibroadenoma	6	7	7
Animals with adenocarcinoma	1	2	5
Total number of mammary tumors	8	13	22

*indicates control group.

Historical control data on mammary tumors in female CD Sprague Dawley and fisher 344 rats are summarized in table 2 and table 3 shows historical control data for mammary fibroadenomas, adenoma and adenocarcinoma for female CD sprague Dawley rats.

D. RECOMMENDATION

N.A.I.

/S/
Herman M. Rhee, Ph. D.

/S/
3/18

cc: Original IND, HFD-510
A. Jordan/H. Rhee

APPEARS THIS WAY
ON ORIGINAL

Appendix 2.2

TABLE 3.
HISTORICAL CONTROL DATA FOR MAMMARY FIBROADENOMAS, ADENOMA AND ADENOCARCINOMA FOR FEMALE [redacted] CD SPRAGUE DAWLEY RATS FROM TWELVE 52 WEEK TOXICITY STUDIES.

Study Code	A	B	C	D	E	F	G	H	I	J	K	L
Rats having tissue examined by microscopy	20	22	20	20	24	15	20	20	20	15	20	20
Rats bearing mammary tumours	0	3	0	0	1	2	2	0	1	1	5	1
Rats with fibroadenomas	0	2	0	0	0	0	0	0	0	0	5	1
Rats with adenomas	0	1	0	0	0	0	0	0	1	0	0	0
Rats with adenocarcinomas	0	1	0	0	1	2	2	0	0	1	0	0
Rats with >1 mammary tumour	0	1	0	0	0	0	0	0	0	0	1	0
Total number of mammary tumours in group	0	4	0	0	1	2	2	0	1	1	6	1

Historical data from [redacted] (collected over the last few years).

General Pharmacology Studies Overview

TEST	INSULIN ASPART NEW PROCESS/ HUMAN INSULIN(HI)	INSULIN ASPART OLD PROCESS	RESULTS OF NEW PROCESS INSULIN ASPART
Irwin Observation Test	1,10 or 100 U/kg IV, (P-8) HI 100 IU/kg IV		No difference from human insulin was observed
Locomotor Activity, rats	1,10 or 100 U/kg IV,(P-9) HI 100 IU/kg IV		No consistent effect
Rotarod Performance, mice	1,10 or 100 U/kg IV,(P-10) HI 100 IU/kg IV		No effects

Hexobarbital induced sleeping time, mice	1,10 or 100 U/kg i.v. (P-11) HI 100 IU/kg IV	0.1 or 1.0 U/kg IV, (15487)	No difference from human insulin was observed
Ethanol induced sleeping, time mice	1,10 or 100 U/kg IV, (P-12) HI 100 IU/kg IV	0.1 or 1.0 U/kg IV, (15587)	No difference from human insulin was observed
Anti-convulsant activity, mice	1,10 or 100 U/kg IV, (P-13) HI 100 IU/kg IV		No effects
Pro-convulsant activity, mice	1,10 or 100 U/kg IV, (P-14) HI 100 IU/kg IV		No effects
Analgesic effect on acetic acid induced writhing	1,10 or 100 U/kg IV, (P-15) HI 100 IU/kg IV		No effects
Effects on body temperature	1,10 or 100 U/kg IV,(P-16) HI 100 IU/kg IV		No effects
Isolated guinea-pig ileum	3.6, 36 or 360 mU/ml (P-17) HI: 360 mIU/ml	0.001, 0.01 or 0.1 U/ml (16987)	No effects
Autonomic nervous system in anaesthetized cat	0.4, 1.0 and 4.0 U/kg IV, (P-18) HI: 0.4, 1.0 and 4.0 IU/kg IV	0.7 and 0.8 U/kg IV. (15787)	No difference from human insulin was observed
Cardiovascular and Respiratory Systems in anaesthetized rat	1,10 and 100 U/kg IV, (P-19) HI: 1,10 and 100 IU/kg IV	0.07 and 0.079 U/kg IV. (15687)	No effects
Cardiovascular and Respiratory Systems in anaesthetized cat	0.4, 1.0 and 4.0 U/kg IV, (P-18) HI: 0.4, 1.0 and 4.0 IU/kg IV	0.07 and 0.8 U/kg IV (15787)	No difference from human insulin was observed
Cardiovascular and Respiratory Systems in anaesthetized pig	0.4, 1.0 and 4.0 U/kg IV. (P-20) HI: 0.4, 1.0 and 4.0 IU/kg IV	0.09 U/kg and 0.9 U/kg IV(15887)	No difference from human insulin was observed

Gastrointestinal Motility in Mice	1,10 or 100 U/kg IV, (P-21) HI 100 IU/kg IV		No effects
Renal Function in Rats	1,10 or 100 U/kg IV, (P-22) HI 100 IU/kg IV	0.0U/kg or 0.77U/kg IV (15087)	No effects in general

Summary of treatment changes

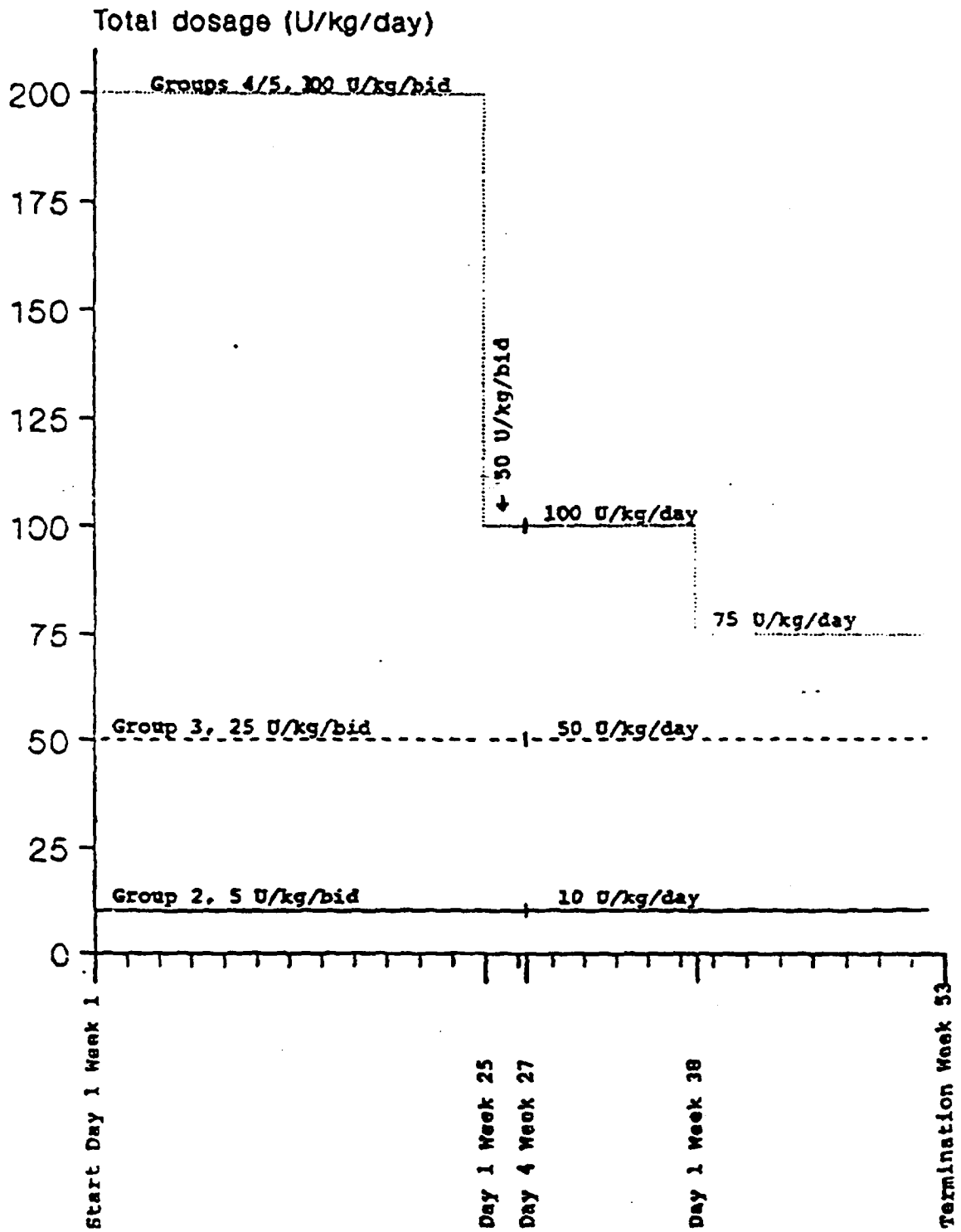

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Figure 9. Plasma concentrations of Insulin X14 or human insulin 1 hour after the first daily dose after Insulin X14 doses of 10, 50 and 200 →100 →75U/kg and HM(ge) doses of 200 →100 →75IU/kg and control as function of time during 52 Weeks Toxicity Study in Rats

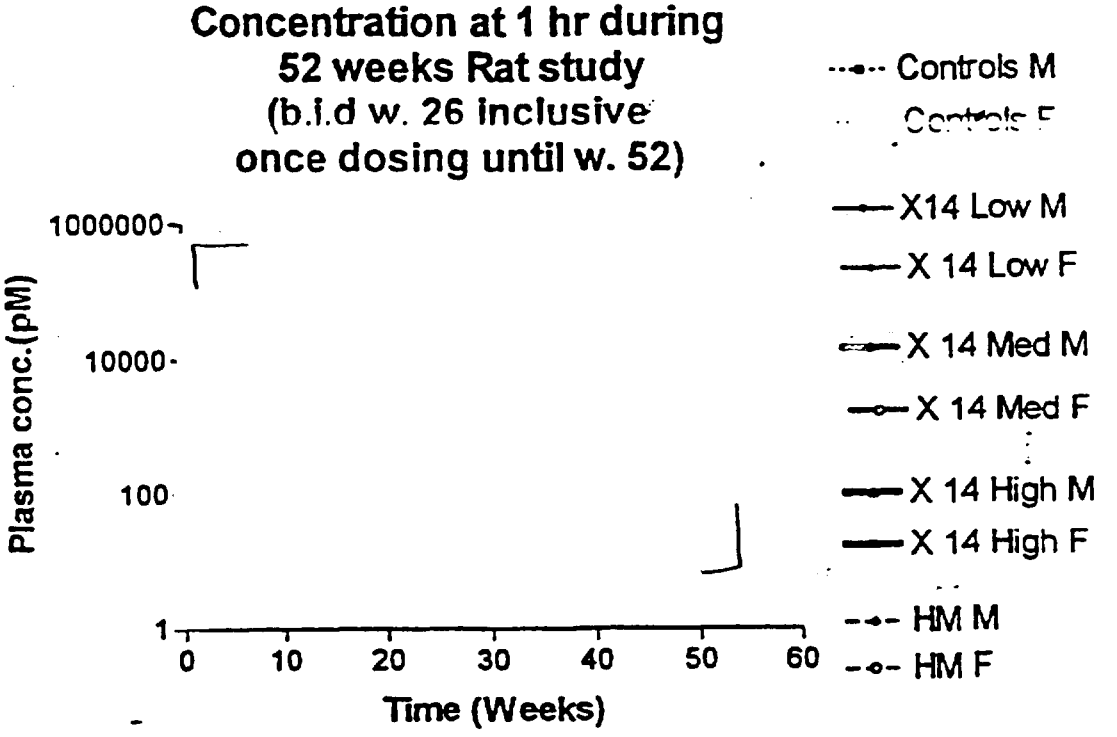

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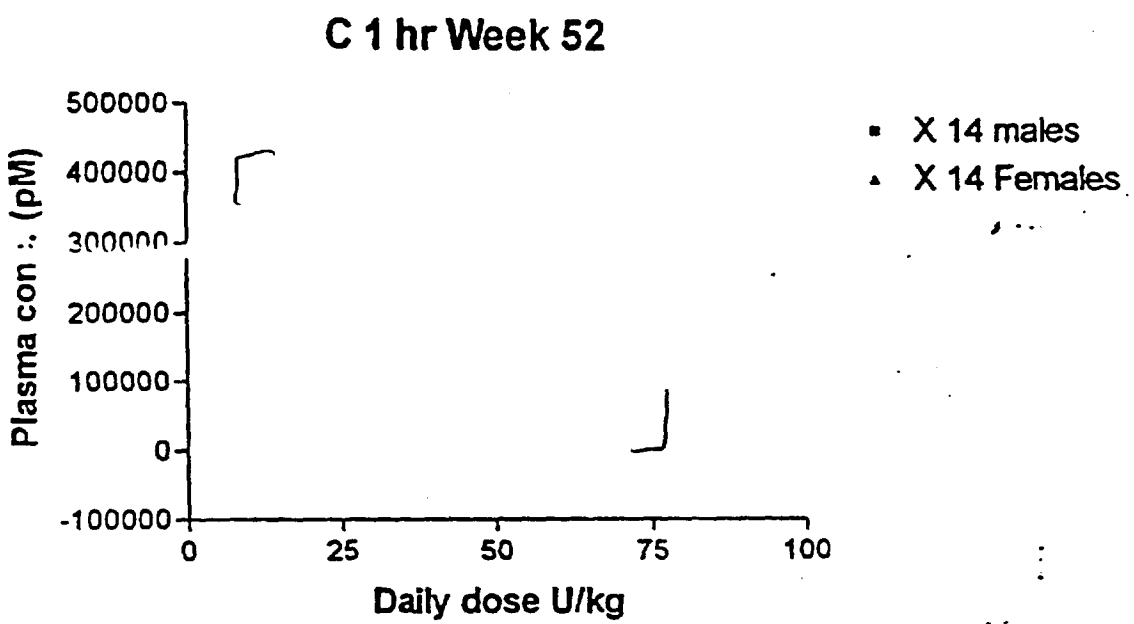
Figure 8. Insulin X14 plasma concentrations 1 hour after the first daily dose $C_{1\ hour}$ values as function of Insulin X14 dose in Week 52 of 52 Weeks s.c. Toxicity Study in Male and Female Rats



Results of regression analysis

Males:

Slope: [redacted], Y-intercept : [redacted]

P = 0.0001 for slope different from zero,

P = 0.1424 for departure from linearity.

Females:

Slope: [redacted] Y-intercept : [redacted]

P = 0.0054 for slope different from zero,

P = 0.6492 for departure from linearity.

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Figure 4. Mean (± SD) Insulin X14 and Human Insulin Plasma Profiles for Male and Female Rats (n = 3 - 4) in Week 52 of 52 Weeks s.c. Toxicity Study. Endogenous rat insulin in controls were measured as human insulin

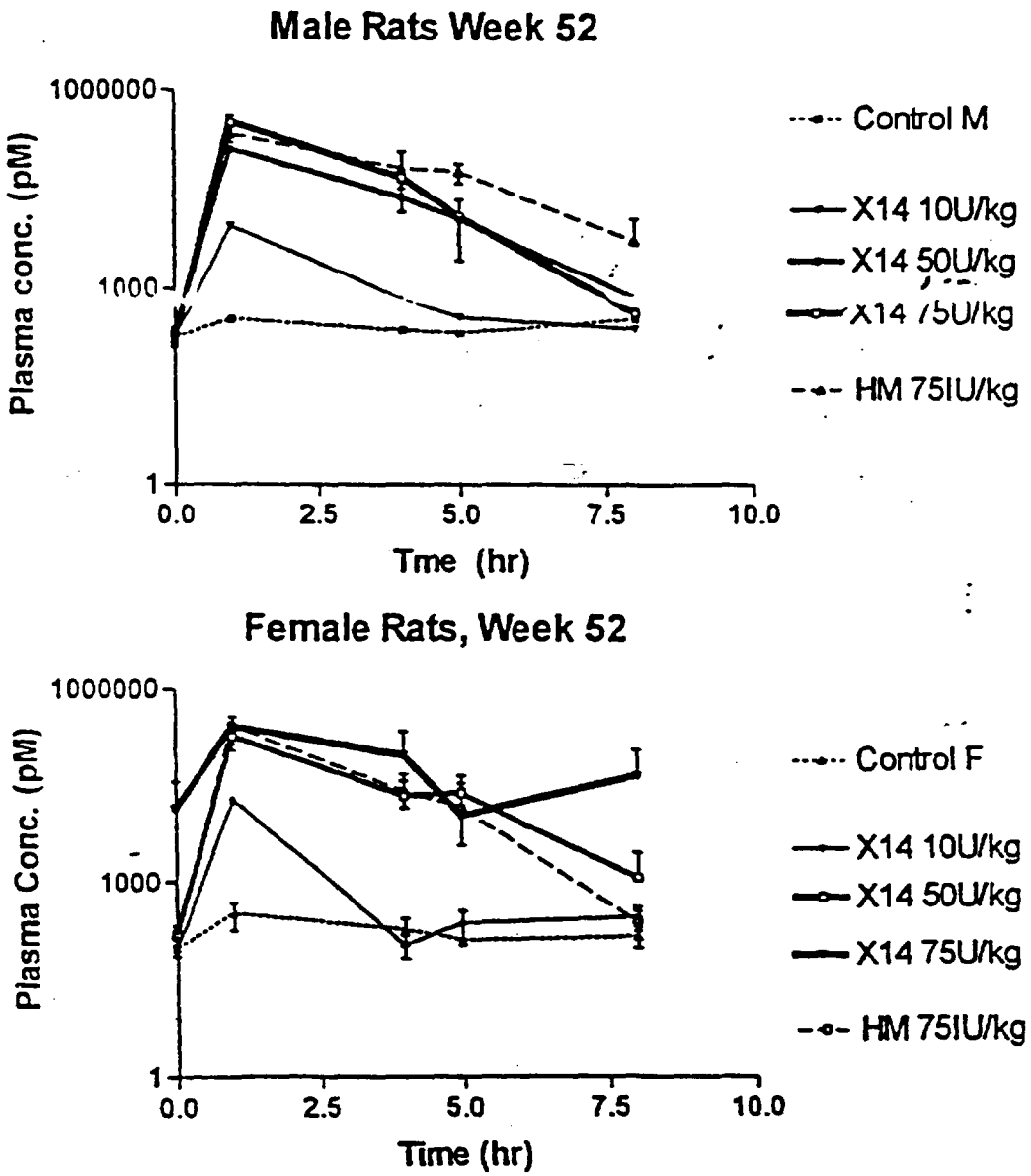

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Figure 4. Mean (± SD) Insulin X14 and Human Insulin Plasma Profiles for Male and Female Rats (n = 3 - 4) in Week 52 of 52 Weeks s.c. Toxicity Study. Endogenous rat insulin in controls were measured as human insulin

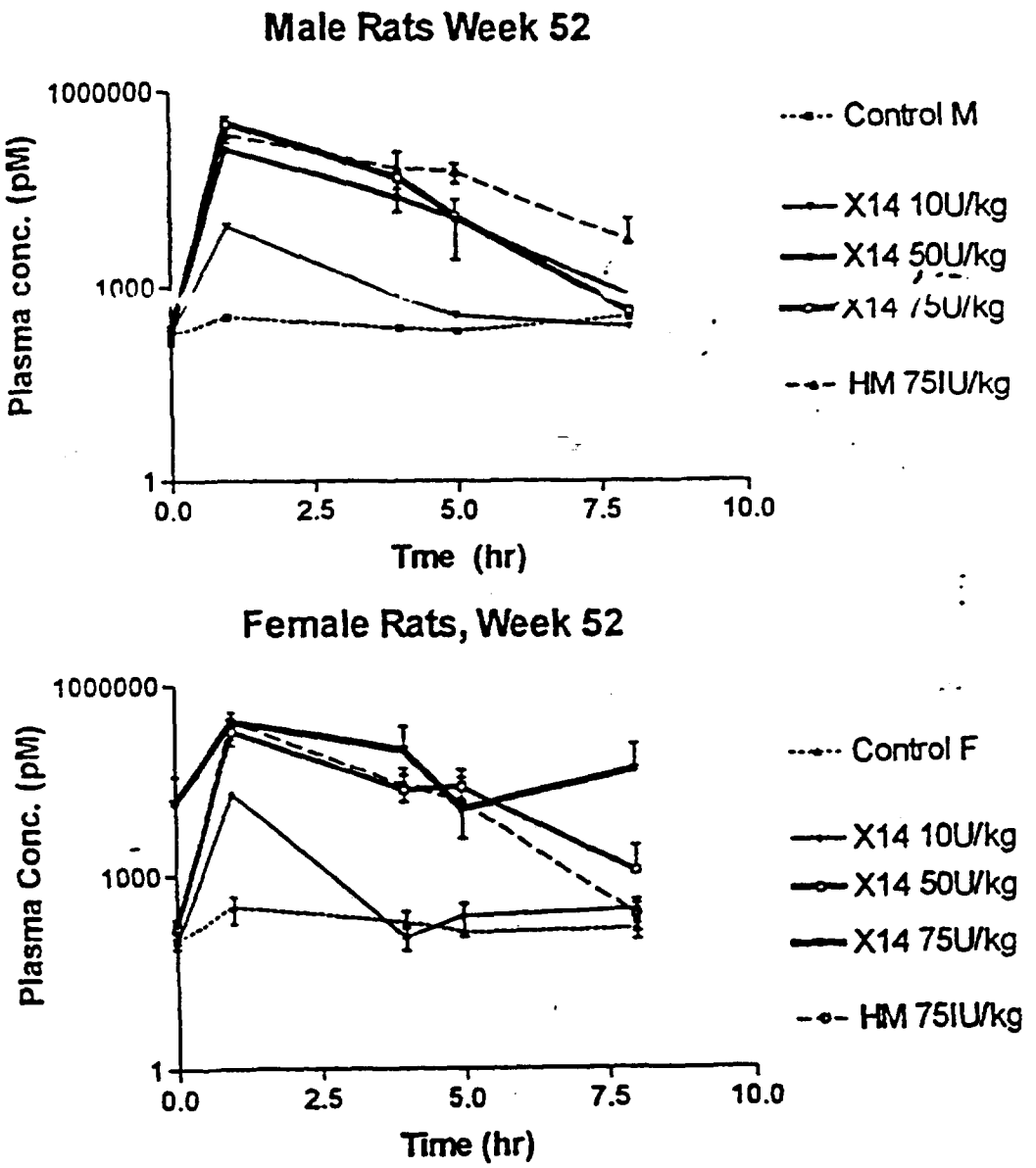

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Figure 11. C_{1hour} values after Insulin X14 dosed 0.5, 1 and 2 U/kg and HM(ge) (2IU/kg) and control with time during 52 Weeks Toxicity Study in Beagle Dogs.

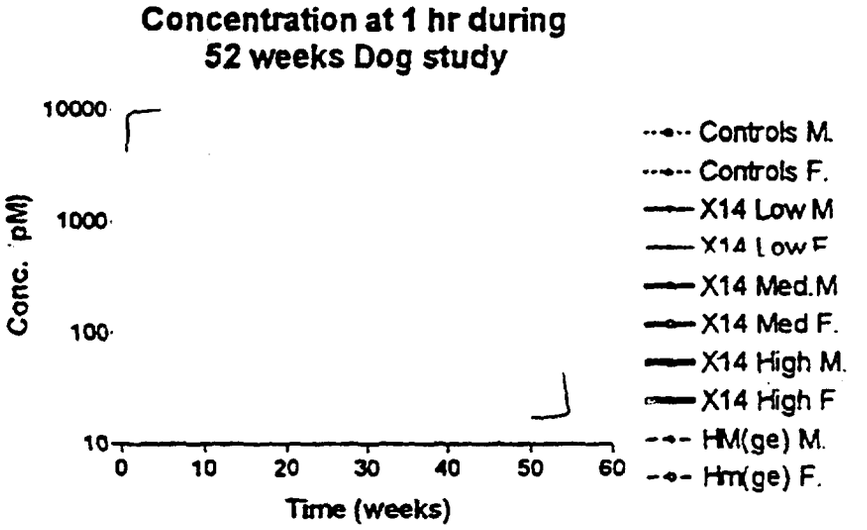

Figure 10. $C_{1\ hour}$ values as function of Insulin X14 dose in Week 52 of 52 Weeks s.c. Toxicity Study in Male and Female Beagle Dogs.

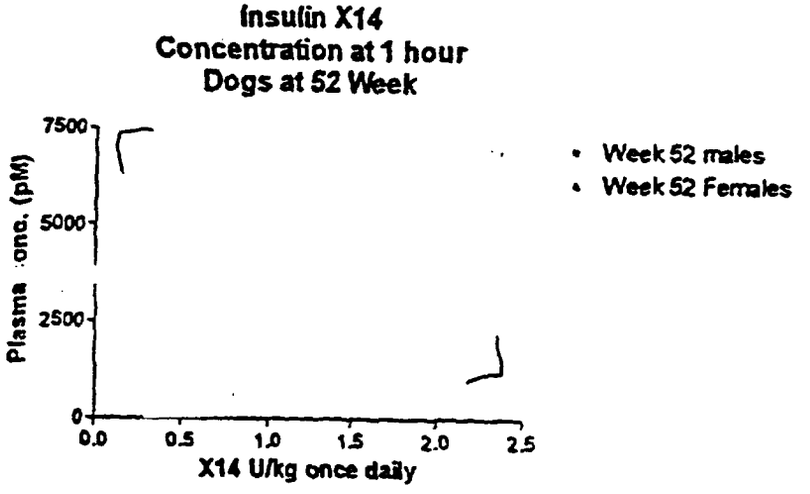

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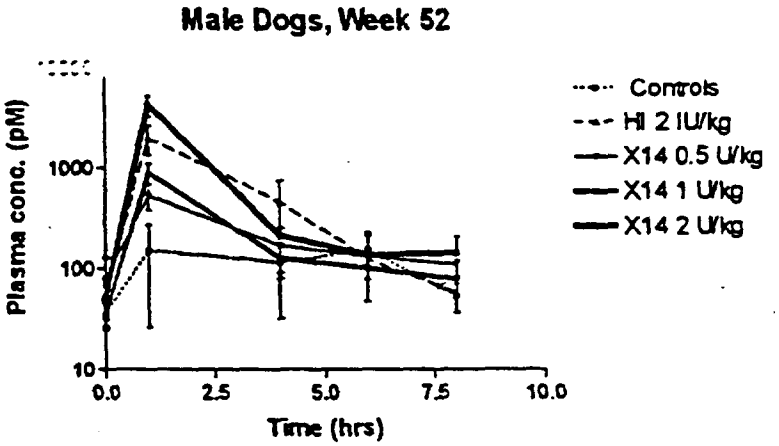
Figure 5. Mean (± SD) Insulin X14 and Human Insulin Plasma Profiles for Male and Female Beagle dogs (n = 4) in Week 52 of 52 Weeks s.c. Toxicity Study. Endogenous canine insulin in controls were measured as human insulin



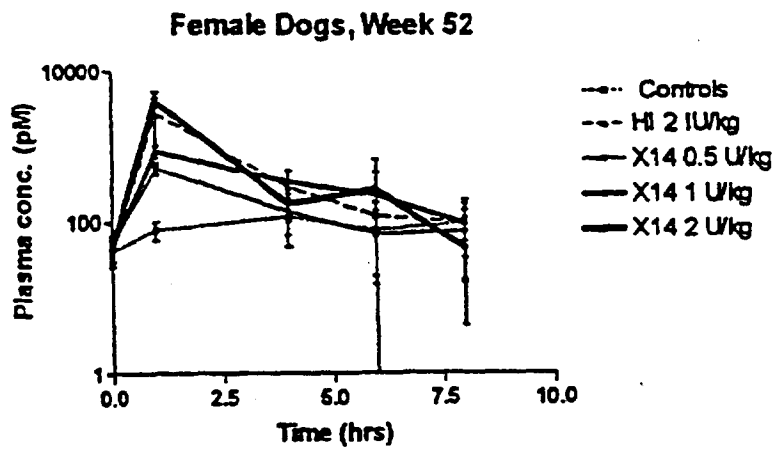


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Table 1 Mean concentrations of radioactivity in tissues following subcutaneous administration of ^{125}I(Tyr A14)-X14 to male rats

Tissue	0.5 Hours	2 Hours	4 Hours	24 Hours	168 Hours
	Mean ± sd δ	Mean ± sd δ	Mean ± sd δ	Mean ± sd δ	Mean ± sd δ
Whole-blood	7.06 ± 0.352	6.38 ± 0.468	5.48 ± 0.144	1.50 ± 0.280	0.126 ± 0.0159
Plasma	8.73 ± 0.592	7.64 ± 0.780	6.27 ± 0.289	2.05 ± 0.422	0.211 ± 0.0221
Blood cells	5.25 ± 0.286	5.53 ± 0.376	4.73 ± 0.160	0.725 ± 0.106	0.0392 ± 0.0100
Injection site	78.3 ± 3.84	15.6 ± 2.73	15.0 ± 3.66	3.99 ± 1.35	3.39 ± 1.23
Adrenal glands	5.41 ± 0.612	2.48 ± 0.0265	2.13 ± 0.859	1.65 ± 1.82	0.374 ± 0.416
Aorta	8.72 ± 1.41	12.0 ± 2.24	9.02 ± 1.57	2.55 ± 1.09	0.558 ± 0.291
Bladder	7.93 ± 5.78	8.75 ± 7.39	5.21 ± 1.80	1.29 ± 0.641	0.223 ± 0.241
Bone	1.30 ± 0.556	1.89 ± 0.832	1.83 ± 0.550	0.258 ± 0.112	0.0496 ± 0.0276
Bone marrow	4.05 ± 0.376	3.58 ± 0.163	3.06 ± 0.157	2.23 ± 3.06	0.0760 ± 0.0660
Brain	0.816 ± 0.0995	0.445 ± 0.117	0.328 ± 0.119	0.143 ± 0.0377	0.0432 ± 0.0189
Caecum wall	4.17 ± 1.55	9.76 ± 4.14	13.0 ± 2.54	1.38 ± 0.460	0.130 ± 0.0461
Eyes	2.08 ± 0.357	2.47 ± 0.118	2.01 ± 0.101	0.392 ± 0.0365	0.0392 ± 0.0091
Fat (brown)	20.0 ± 34.9	2.46 ± 0.320	2.40 ± 0.211	0.584 ± 0.189	0.0643 ± 0.0235
Fat (white)	0.877 ± 0.158	0.650 ± 0.117	0.649 ± 0.108	0.182 ± 0.0165	0.0372 ± 0.0105
GIT contents	7.27 ± 2.03	22.7 ± 2.36	21.8 ± 1.82	2.98 ± 0.311	0.228 ± 0.0474
Heart	5.08 ± 0.241	2.94 ± 0.226	2.04 ± 0.0800	0.580 ± 0.108	0.0580 ± 0.0051
Kidneys	43.5 ± 3.32	10.1 ± 1.18	4.50 ± 0.137	1.17 ± 0.0153	0.171 ± 0.0132
Lacteryeal glands	4.54 ± 0.516	3.03 ± 0.321	2.25 ± 0.0458	0.562 ± 0.126	0.120 ± 0.115
LI wall	6.79 ± 3.50	12.1 ± 3.48	17.9 ± 6.91	0.973 ± 0.181	0.152 ± 0.0031
Liver	6.27 ± 0.367	2.72 ± 0.199	2.40 ± 0.170	0.947 ± 0.198	0.115 ± 0.0070
Lung	5.33 ± 0.338	5.77 ± 2.68	3.44 ± 0.110	0.846 ± 0.145	0.102 ± 0.0079
Lymph nodes	3.56 ± 0.520	3.36 ± 0.319	2.61 ± 0.258	0.616 ± 0.129	0.0481 ± 0.0102
Muscle	2.36 ± 0.249	1.53 ± 0.107	0.965 ± 0.0361	0.248 ± 0.0404	0.0305 ± 0.0087
Pancreas	10.5 ± 0.685	4.44 ± 0.223	2.73 ± 0.136	0.612 ± 0.0920	0.0555 ± 0.0026
Pituitary	5.23 ± 1.35	2.94 ± 0.826	3.01 ± 0.679	nd	nd
Prostate	2.71 ± 0.454	7.32 ± 7.18	4.28 ± 1.13	0.754 ± 0.280	0.0367 ± 0.0035
Salivary glands	8.23 ± 0.220	4.22 ± 0.465	3.29 ± 0.131	0.606 ± 0.0781	0.0510 ± 0.0048
Skin*	4.41 ± 0.561	5.65 ± 0.870	5.76 ± 0.410	1.52 ± 0.441	0.208 ± 0.0437
SI wall	7.21 ± 1.69	17.7 ± 2.21	16.5 ± 3.61	1.88 ± 0.140	0.127 ± 0.0059
Spleen	4.05 ± 0.356	2.92 ± 0.223	2.43 ± 0.0751	0.436 ± 0.0699	0.0402 ± 0.0118
Stomach wall	14.2 ± 3.32	48.0 ± 12.8	56.5 ± 16.9	4.04 ± 0.341	0.156 ± 0.0342
Testes	1.41 ± 0.222	2.18 ± 0.159	2.40 ± 0.0833	0.430 ± 0.0634	0.0322 ± 0.0037
Thymus	2.53 ± 0.214	2.07 ± 0.199	1.77 ± 0.0721	0.332 ± 0.0697	0.0356 ± 0.0037
Thyroid	288 ± 33.1	2440 ± 436	7080 ± 989	20700 ± 5280	5260 ± 1920
Trachea	3.28 ± 0.201	4.42 ± 0.317	10.3 ± 2.37	11.1 ± 13.2	0.739 ± 0.402
Vena cava	10.8 ± 1.57	11.8 ± 2.46	11.8 ± 3.28	28.3 ± 25.1	1.14 ± 1.11

Results are expressed as the mean ($n = 3$) radioactivity concentration in pmol equivalents/g tissue

GIT gastrointestinal tract
LI large intestine
SI small intestine
sd standard deviation
nd no radioactivity detected
* remote from the injection site

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Table 4 Mean concentrations of radioactivity in tissues following subcutaneous administration of ^{125}I(Tyr A14)-X14 to female rats

Tissues	0.5 Hours	2 Hours	4 Hours	24 Hours	168 Hours
	Mean ± sd †	Mean ± sd †	Mean ± sd †	Mean ± sd †	Mean ± sd †
Whole-blood	8.59 ± 0.763	9.78 ± 0.852	6.90 ± 1.59	0.965 ± 0.0976	0.136 ± 0.0632
Plasma	10.9 ± 0.798	11.4 ± 1.01	8.09 ± 1.84	1.46 ± 0.401	0.208 ± 0.0795
Blood cells	5.83 ± 0.755	8.39 ± 0.660	5.72 ± 1.40	0.423 ± 0.0300	0.0254 ± 0.0287
Injection site	79.6 ± 16.2	14.9 ± 6.40	8.11 ± 2.43	1.20 ± 0.0513	0.221 ± 0.0581
Adrenal glands	4.88 ± 0.412	4.24 ± 0.718	2.41 ± 0.307	1.21 ± 1.08	0.398 ± 0.294
Aorta	12.4 ± 1.35	13.6 ± 3.08	11.1 ± 1.47	1.60 ± 0.165	0.393 ± 0.107
Bladder	6.34 ± 0.783	9.52 ± 2.49	8.39 ± 4.46	0.562 ± 0.103	0.0441 ± 0.0411
Bone	1.73 ± 0.546	2.11 ± 0.385	2.04 ± 0.709	0.142 ± 0.0286	0.0246 ± 0.0259
Bone marrow	3.86 ± 3.41	5.18 ± 0.615	5.06 ± 0.755	1.86 ± 1.43	nd
Brain	0.814 ± 0.0365	0.609 ± 0.141	0.453 ± 0.0743	0.132 ± 0.0241	0.0290 ± 0.0121
Caecum wall	4.48 ± 0.711	13.9 ± 5.49	7.90 ± 0.891	0.716 ± 0.105	0.138 ± 0.0095
Eyes	2.51 ± 0.153	3.29 ± 0.187	2.41 ± 0.545	0.321 ± 0.0629	0.0380 ± 0.0131
Fat (brown)	9.20 ± 6.75	5.14 ± 0.168	2.24 ± 0.288	0.340 ± 0.0575	0.0564 ± 0.0156
Fat (white)	1.03 ± 0.302	0.831 ± 0.164	0.566 ± 0.0550	0.0911 ± 0.0143	0.0254 ± 0.0075
GIT contents	7.02 ± 0.666	19.9 ± 3.96	19.1 ± 1.87	2.05 ± 0.336	0.235 ± 0.0382
Heart	5.24 ± 0.464	3.95 ± 0.464	2.44 ± 0.481	0.369 ± 0.0536	0.0803 ± 0.0212
Kidneys	41.3 ± 7.06	11.1 ± 1.20	5.32 ± 0.809	0.835 ± 0.101	0.158 ± 0.0427
Lachrymal glands	5.77 ± 0.569	4.31 ± 0.385	2.98 ± 0.548	0.389 ± 0.0796	0.0666 ± 0.0388
LI wall	6.04 ± 1.38	18.1 ± 5.23	13.9 ± 2.19	0.913 ± 0.304	0.128 ± 0.0167
Liver	6.92 ± 0.489	3.79 ± 0.382	2.80 ± 0.606	1.06 ± 0.212	0.207 ± 0.0525
Lung	6.26 ± 0.197	6.32 ± 0.470	4.46 ± 0.930	0.584 ± 0.0834	0.0980 ± 0.0298
Lymph nodes	3.82 ± 0.574	4.68 ± 0.690	3.52 ± 0.754	0.411 ± 0.0488	0.0610 ± 0.0293
Mammary glands	3.59 ± 0.828	2.90 ± 0.159	2.61 ± 0.657	0.317 ± 0.0904	0.0992 ± 0.0707
Muscle	2.37 ± 0.184	1.66 ± 0.116	1.07 ± 0.152	0.134 ± 0.0131	0.0212 ± 0.0069
Ovaries	5.94 ± 0.352	6.52 ± 0.461	5.85 ± 1.03	0.614 ± 0.0896	0.0776 ± 0.0296
Pancreas	11.4 ± 1.15	5.88 ± 0.735	3.36 ± 0.682	0.392 ± 0.0423	0.0575 ± 0.0193
Pituitary	5.92 ± 0.805	20.6 ± 28.2	4.42 ± 1.02	0.580 ± 0.0396	nd
Salivary glands	10.1 ± 1.20	6.00 ± 0.332	3.98 ± 0.774	0.387 ± 0.0402	0.0569 ± 0.0210
Skin	5.52 ± 1.55	7.32 ± 1.12	5.94 ± 0.797	1.08 ± 0.344	0.465 ± 0.381
SI wall	6.61 ± 1.23	17.9 ± 1.69	16.0 ± 4.26	0.979 ± 0.157	0.130 ± 0.0239
Spleen	4.16 ± 0.227	4.13 ± 0.347	2.93 ± 0.540	0.300 ± 0.0387	0.0370 ± 0.0098
Stomach wall	15.6 ± 1.66	65.7 ± 26.4	50.5 ± 3.79	3.04 ± 0.771	0.122 ± 0.0538
Thymus	3.08 ± 0.293	3.39 ± 0.225	2.09 ± 0.251	0.319 ± 0.154	0.0378 ± 0.0172
Thyroid	338 ± 130	3940 ± 1190	10500 ± 4800	19300 ± 13300	4390 ± 2350
Trachea	2.99 ± 0.835	4.31 ± 1.81	4.97 ± 1.60	20.3 ± 20.1	2.02 ± 1.62
Uterus	6.58 ± 1.97	7.61 ± 0.565	6.59 ± 1.33	0.710 ± 0.168	0.0922 ± 0.0553
Vena cava	9.83 ± 1.70	15.9 ± 7.58	14.3 ± 7.18	5.01 ± 4.55	1.93 ± 0.147

Results are expressed as the mean ($n = 3$) radioactivity concentration in pmol equivalents/g tissue

GIT gastrointestinal tract
LI large intestine
SI small intestine
sd standard deviation
nd no radioactivity detected

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Table 7 **Mean concentrations of radioactivity in tissues following subcutaneous administration of ^{125}I(Tyr A14)-X14 to pregnant female rats**

Tissues	0.5 Hours	2 Hours	4 Hours	24 Hours
	Mean ± sd ♀	Mean ± sd ♀	Mean ± sd ♀	Mean ± sd ♀
Whole-blood	7.81 ± 1.42	9.06 ± 0.733	6.34 ± 1.53	0.667 ± 0.195
Plasma	10.1 ± 1.72	10.7 ± 0.794	6.89 ± 1.61	0.878 ± 0.216
Amniotic fluid	0.320 ± 0.0683	0.630 ± 0.0839	0.513 ± 0.0665	0.0733 ± 0.0268
Brain	0.700 ± 0.0864	0.504 ± 0.0497	0.367 ± 0.158	0.0806 ± 0.0190
Foetuses	0.999 ± 0.220	0.778 ± 0.0849	0.363 ± 0.0950	0.0502 ± 0.0073
Heart	5.21 ± 0.749	3.86 ± 0.354	2.33 ± 0.373	0.261 ± 0.0593
Kidneys	52.2 ± 2.90	9.56 ± 1.57	4.64 ± 0.789	0.681 ± 0.167
Liver	5.93 ± 1.28	3.42 ± 0.0964	2.13 ± 0.477	0.688 ± 0.121
Lungs	6.64 ± 1.07	6.83 ± 0.122	4.72 ± 1.36	0.432 ± 0.0808
Mammary tissue	3.07 ± 0.320	4.64 ± 0.380	4.62 ± 1.06	0.801 ± 0.234
Ovaries	4.26 ± 0.684	4.12 ± 0.442	2.60 ± 0.509	0.345 ± 0.0689
Placentae	3.71 ± 0.857	5.89 ± 0.610	4.09 ± 1.18	0.417 ± 0.0852
Uterus	4.87 ± 1.03	6.61 ± 0.665	4.86 ± 1.16	0.544 ± 0.0270

Results are expressed as the mean (n=3) radioactivity concentration in pmol equivalents/g tissue
sd standard deviation

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Incidence of mammary tumours in female rats

	Control	X14			HM(ge)
Dosage level (U/kg/bid)	**0**	**5**	**25**	**100**	**100**
Animals bearing mammary tumours	7	11	11	11[1]	6
Animals with fibroadenoma(s)/adenoma	6	9	10	8[2]	5
Animals with adenocarcinoma(s)	2	4	2	4	1
Animals with more than one mammary tumour	4	2	2	3	3
Total number of mammary tumours in group	11	13	13	14	13
Animals having tissue examined by microscopy	32	32	32	32	32

[1] $p=0.003$ for the trend test for all mammary tumours
[2] $p=0.039$ for the trend test for benign mammary tumours

Stomach

Erosion of the glandular epithelium was observed in decedent male and female animals from the 100 IU HM(ge)/kg/bid, 25 and 100 U X14/kg/bid groups and one male decedent animal from the 5 U X14/kg/bid group.

Erosion of the glandular epithelium of the stomach in decedent rats

	Control				X14				HM(ge)	
	0		5		25		100		100	
Dosage level (U/kg/bid)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Erosion of the glandular epithelium	0	0	1	0	2	1	8	4	12	5
Total number of stomachs examined	2	3	1	4	4	3	18	18	20	17

This change was not detected in any animal examined at termination.

Pathology and histopathology can be summarised as follows:

Treatment	—	X14	HI	Untreated
Animals total	20	20	20	20
Preliminary deaths	5	3	8	0
Sacrificed for humane reasons	1	2	0	1
One year survivors	14	15	12	19
Animals having tissue examined by microscopy	17	18	17	20
Animals with adenoma(s) in mammary glands	11	7	8	4
Animals with adenocarcinoma(s) in mammary glands	3	4	3	1

The number of fatal adenomas is so small that they show no significant differences by themselves. By combined analysis of fatal and incidental adenomas in the mammary glands, we obtain the following test statistics:

Combined analysis of animals with fatal and incidental adenomas. Separate comparison of each pair of groups					
Preparation 1	Preparation 2	positive 1	expected 1	standard error of residual	p
—	Actrapid	11	9.5	1.4	0.30
X14	Actrapid	7	8.3	1.6	0.39
Untreated	Actrapid	4	7.2	1.4	<0.05
—	Untreated	11	6.6	1.6	<0.01
X14	Untreated	7	4.9	1.3	0.16
—	X14	11	8.9	1.7	0.20

Addendum to the Pharmacologist's July 21, 99 review of NDA 20-986

A. LABELING

Following issues were discussed further on September 10, 1999, after completion of the pharmacology review (dated July 21, 1999):

1. Carcinogenicity Findings: In a 52-week toxicity study in rats (QA certified study), all doses of the drug (X14 at 10, 50 and 200 U/kg/day) caused mammary gland tumors (benign + malignant) compared to vehicle controls (11, 11, 11 respectively vs 7 in controls p=0.003-0.0039), than human insulin (200 U/kg/day) compared to vehicle controls (6 vs 7 in controls p=0.24). This was analyzed by Peto's analysis, which shows that all doses significantly increased the mammary tumors, in the trend test. Also, slightly, but not statistically significant increases (p=0.062) in mammary gland tumors were observed between X14 and regular human insulin (both at 200 U/kg/day) in this study. The mammary gland tumor findings with X14 were above the historical control data.

2. Pregnancy Category: The pre- and post-implantation losses, and visceral/skeletal abnormalities were observed in both rats and rabbits with X14 (at approximately 32 and 3 times the human dose), and CFR indicates that this pregnancy category should be 'C'. Previous studies have shown that insulin given during pregnancy can induce structural changes in the offsprings in various species, this happens at doses as low as 1 U or less (Schardein J.L. in Chemically-induced birth defects, second edition, Marcel Dekker, Inc., New York and Basel). Furthermore, in pregnant rats, brief hypoglycemia with insulin treatment during organogenesis, can disrupt normal embryo development (J. Clin. Invest. 78: 643, 1986).

Based on these discussions, we have made additional labeling modifications (see attached label).

Carcinogenicity, Mutagenicity, Impairment of Fertility

[ ]

Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of ——. In a 52-

week study , dosed subcutaneously with at 10, 50 and 200 U/kg/day (approximately 2, 8 and 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area)

was not genotoxic[104] in the following tests, Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and in ex vivo UDS test in rat liver hepatocytes. In fertility studies in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed[105].

"Pregnancy- Teratogenic Effects-Pregnancy Category C'

[ ]

Subcutaneous reproduction and teratology studies have been performed with and regular human insulin in rats and rabbits. In these studies was given to female rats before mating, during mating and throughout pregnancy, and to rabbits during organogenesis. These effects of did not differ from those with subcutaneous regular human insulin caused pre- and post-implantation losses, and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32-times the human subcutaneous dose, based on U/body surface area), and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose, based on U/body surface area). No significant effects were observed in rats at a dose of 50 U/kg/day and rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times human subcutaneous dose,

[ ]

[redacted]

Nursing- It is unknown whether [redacted] is excreted [redacted] in human milk.

[redacted]

The justification for the changes are as follows:

1. Carcinogenicity: The mammary gland tumor findings with X14 were greater than historical controls, and slightly greater than insulin. We believe this should be in the label to describe the findings. Slightly, but not statistically significant increases (p=0.062) in mammary gland tumors were observed between X14 and regular human insulin (at 32-times the human dose) in a 52-week toxicity study in rats (QA certified study). X14 also had a higher potential in promoting benign and combined (benign + malignant) mammary gland tumors compared to vehicle controls (p=0.003-0.0039), than human insulin compared to vehicle controls (p=0.24). However, X14 is not genotoxic. Therefore, under 'Carcinogenicity', the reviewer is suggesting the above text for labeling.
2. Pregnancy: Studies in rats were conducted up to 32-times the human dose, and in rabbits up to 3-times the human dose respectively, under [redacted] -Pregnancy', the reviewer is suggesting a change in title and the text for labeling. Since pre- and post-implantation losses, and visceral/skeletal abnormalities were observed in both rats and rabbits with X14 (at approximately 32 and 3 times the human dose), and even though these were similar to insulin, technically according to CFR, this makes it a pregnancy category 'C'. Insulin has no pregnancy category labeling, so there

is no clear comparison. Lys-pro (another analog) has category B in pregnancy labeling, since it had no findings (but it was only tested at 4 and 0.3 times the human dose in rats and rabbits respectively)

B. In pharmacologist's review of July 21, 99: Under overall summary and evaluation: other clinically relevant issues- the second 1-year toxicity study in rats.

The last sentence from this review on page 69 which states that

[' is deleted from the review. This is due to the fact that mammary tumors were observed in this study with all doses of the drug X14 (10, 50, and 200 U/kg/day).

C. Segment III reproductive Toxicity study

In the segment III toxicity studies in NDA 20-986 (submitted on 4/19/99, study # 940304), following is now added. The macroscopic examinations in segment III study showed that F1 offsprings had a slightly higher incidence of pups with minor kidney changes (mainly increased pelvic dilatation or pale coloration/enlarged kidneys) among litters derived from F0 females. This was found mainly in the right kidneys. The incidences were 1, 4, 7, 3 and 12 at 0, 10, 50, 200 U/kg/day of X14 and 200 U/kg/day with recombinant human insulin respectively. Therefore, incidences were higher with human insulin then with X14. Thus, minor kidney changes were noted with both, X14 and regular human insulin.

D. One-year toxicity studies in rats and dogs

An increase in alkaline phosphatase was observed in clinical studies by the medical reviewer, this was re-examined in animal toxicity studies. In 1 year toxicity study in rats, slight but not significant increases in alkaline phosphatase (ALP) were observed with X14 vs controls in week 51 (males 148-150 at 10-200 U/kg/day vs 136 mu/ml in controls, females 72-91 vs 74 mu/ml in controls). However, a significant increase in ALP was noted with recombinant human insulin at 200 U/kg/day in male rats in this study in week 51 (175* vs 136 mu/ml in controls, *$p<0.05$), but not in female rats (91 vs 74 mu/ml in controls).

In a 1 year dog toxicity study, slight increases in alkaline phosphatase (ALP) were also observed with X14 vs controls in week 52 (133 at 2 U/kg/day vs 99 mu/ml in controls). Similar slight increases in ALP were also noted with recombinant human insulin at 2 U/kg/day in dogs in this study in week 52 (112 vs 99 mu/ml in controls).

In summary, ALP tended to be slightly higher in X14 than in vehicle control groups, but it was also higher in animals treated with regular human insulin, and significance of this finding is unclear. In human studies ALP was significantly elevated in two studies.

/S/ 9/14/99

Indra Antonipillai
Pharmacologist, HFD-510

Team Leader concurs
/S/
9/14/99

cc: NDA Arch
HFD510
HFD510/antonipillai/steigerwalt/koller/jrhee
Filename: ———

APPEARS THIS WAY
ON ORIGINAL