Table 12. Skeletal and visceral abnormalities in segment II toxicity study in rabbits:

	Cont	X14			HM(ge)		
Dosage (U/kg/bid)	0	0.5	1.5	5	0.5	1.5	5
Visceral/Skeletal Abnormalities (FA/LA)							
Litters examined	18	17	19	17	17	18	20
Fetuses examined	145	138	160	100	152	152	133
Visceral: Thoracic							
Absent intermediate lung lobe	3/1	0/0	4/4	3/2	4/1	2/2	0/0
Lumbar/abdominal							
Gall bladder-small/bilobed/bifurcated	1/1	1/1	4/3	1/1	6/3	2/2	2/1
Abnormal lobation liver	0	1/1	2/2	2/1	3/3	2/2	0/0
Skeletal Abnormalities (FA/LA)	20/ 13	30/ 14	33/ 13	30/ 13	24/ 8	36/ 15	34/ 15
Rib abnormalities: Absent (FA)	1	1	2	4	0	0	3
Fused/connected	0	0	3	7	0	2	6
Sternum: Fused/connected centers	1	2	3	3	2	1	3
Vertebral: scoliosis	0	0	3	2	0	0	2
Total vertebral/rib/sternal defects	5/3	9/8	10/6	19/ 11	5/4	10/9	13/7
Reduced ossification:cervical	5	10	14	4	1	3	7
Thoracic:	3	2	2	7	0	0	2
sacrocaudal	0	1	1	1	0	0	0
Cranial: Sutural bones	1	3	4	3	1	2	3
Irregular ossification of cranial bones	1	4	2	2	1	5	5
Thoracic-irregular costal cartilage	0	1	2	0	1	0	1
One less thoracolumbar vertebra	1	2	0	2	1	1	3

FA= Fetuses affected, LA= Litters affected

Toxicokinetics (Study # 960419, test facility Novo Nordisk Park, Malov): Highest insulin X14 levels were generally noted at 1 hr and 5 hrs. Dose linearity was generally noted.

The mean values for groups were (nM):

Groups	X14	HM(ge)
1-hr	1.39, 4.56, 12.98	0.85, 6.03, 23.63
5-hr	1.49, 4.93, 18.83	1.17, 4.38, 17.19

The results indicate that X14 given to female rabbits subcutaneously from day 7 to day 18 of gestation, at doses of 0. 0.5, 1.5 and 5.0 U/kg/bid, (or HM(ge) at 0.5, 1.5, and 5

U/kg/bid), caused anabolic/hypoglycemic effects (all doses reduced blood glucose levels). One animal died at 5 U/kg/bid of X14, due to hypoglycemia. Similarly one animal at 1.5 U/kg/bid of HM(ge) also died. Mid-high doses (1.5-5 U/kg/bid) of X14 or HM(ge) increased body weight gains (by 59-68% & by up to 44% resp), food consumptions (by 15-22%), caused effects on litters (including pre- and post implantation loss, abortions and skeletal abnormalities (mainly affecting the axial skeleton). Sponsor states that all the effects are due to insulin -induced hypoglycemia including on fetuses. The response in general was similar to that seen with HM(ge).

Study title: **Segment III study. Effects of Subcutaneous X14 (Twice Daily) On Pre- and Post-natal Development in Rats** (Study # 940304)

Study No: 940304
Site and testing facility, study initiation-completion: ——— ——— 11/18/94-10/18/96.
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: X14 06694 (———), 06994 (———), 07194 (———)
HM(ge) 06294 (———)

METHODS:
Species/strain: Sprague-Dawley rats (Crl:CD BR VAF/plus strain), females 8-10 weeks old, 174-249 g .
Doses employed: 0, 5, 25 and 100 U/kg/bid, 4 hrs apart (or total doses 0, 10, 50 and 200 U/kg/day)
Route of Administration: Subcutaneous
Number of animals/sex/dosing group: 28
Study Design: This study examined the effects of X14 on pre and post natal development in rats. Also a group was included to receive recombinant human insulin HM(ge) at 100 U/kg/bid (total dose 200 U/kg/day) for comparative purposes. Five groups of 28 mated female rats were given the three doses of the drug (at 0, 5 25, and 100 U/kg/bid), and the fifth group received HM(ge) subcutaneously, twice daily (4 hrs apart), from days 6 post coitum to day 20 of lactation (post partum), treatment for 1 more day continued after day 20 post coitum after the birth of young. Control animals received the vehicle (0.16% phenol, 0.172% m-cresol and 1.60 %, and 0.19% zinc). All adult females were allowed to litter and rear their young. At weaning offspring were selected and kept untreated for post weaning development (e.g sexual maturation, performance, behavior, and reproductive capacity). Excess females and pups were sacrificed shortly day 21 post partum and examined externally and internally for abnormalities.

Parameters and endpoints evaluated:
Clinical signs : Daily

Body weights/food consumptions: Body weights and food consumptions were recorded on day 0 (post coitum), 2, 4, 6, 8, 10, 14, 16, 18 and day 20 of pregnancy, and on day 0 of parturition and on days 1, 7, 14 and 21 post partum.
Water consumption; daily up to day 20 PC and again beginning day 1 post partum.
Plasma Glucose: On day 6 PC (the first day of treatment), at 0, 1 and 4 hrs after the first dose.
F0 Generation: Duration of pregnancy was calculated for adults (as the time between mating and the day on which pups were first seen). At delivery, the pups were recorded, examined for malformations, sexed and weighed on days 4, 8, 12, 16 and 21 PP and examined for clinical signs. Dead young were subjected to autopsy. Pups were examined for surface righting reflex, auditory startle reflex (for sensory and motor coordinating systems from day 1 and 11 resp), air righting reflex and pupils reflex (for auditory and visual functioning resp from day 14 and on day 20 resp). After day 21 PP, 24 male and female pups/group were used to form F1 adult generation, the rest were sacrificed and received the terminal examination, the uteri were visually examined for implantation sites.

F1 Generation animals: These were observed for clinical signs, body weights, sexual maturation, developmental/behavioral examination and accelerating rotarod test. When these rats were 84 days old, 1 male and 1 female from the same dose group were mated. F1 dams were observed and weighed on days 0, 7, 14 and 21 days PP. The F1 dams, F2 pups and F2 litters were evaluated as described earlier. F1 adults were sacrificed and subjected to necropsy/internal-external examinations.

Statistical evaluations: Analysis of variance followed by intergroup comparisons were performed on BW, food and water consumptions, duration of pregnancy, litter data, pre weaning and post weaning development data, and sexual maturation data.

RESULTS:
Mortality and Clinical signs: Three animals died at 25, and three at 100 U/kg/bid dose of X14 (these were deaths due to hypoglycemia). There were 8 deaths with HM(ge) at 100 U/kg/bid. Most of these deaths were associated with hypoglycemic episodes in the peri or post natal period, occurring on the same day or up to 5 days later. Therefore treatment was withheld after day 20, until day 1 post partum.

Body weight: In females, during days 8 to 14, the body weight gains increased at 100 U/kg/bid of X14 or HM(ge) (on day 14 these were 50.3, 53.9, 50.2, 55.3*, 58.4* g resp, *$p<0.05$-0.01). These increases generally persisted (compared to controls) till termination day i.e. at birth. These were also observed with HM(ge), but by end of lactation, these had returned to control levels.

Food and water consumption: In females, food/water consumptions were increased during treatment on days 6 to the first week of lactation at high doses of X14 and HM(ge), but food consumption returned to control values during the last two weeks of lactation with both X14 and HM(ge). Water intake was also increased at 25 U/kg/bid

Plasma Glucose Concentrations: There was a reduction in glucose conc at 1 hr, which returned to normal at 4-hrs with 5 or 25 U/kg/bid of X14. However at 100 U/kg/bid at X14 or with HM(ge), the low glucose conc persisted.

On day 6 post coitum:	1-hr	4-hrs
with X14 and HM(ge):	9.5, 5.2, 4.5, 5.2, 5.0	9.4, 9.5, 9.3, 5.1, 4.9

Duration of pregnancy: Duration of pregnancy was increased ($p<0.05$) at 25 and 100 U/kg/bid of X14 or human insulin (15, 19, 20, 25 and 23 days at 0, 5, 25, 100 and 100 U/kg/bid of X14 and HM(ge) resp)

Fo generation animals: One female from control and at 25 U/kg/bid groups, gave birth to a litter of entirely dead pups, this was not observed at higher doses. At 25 and 100 U/kg/bid of the drug or human insulin, 3, 3, and 6 litters resp were killed following the death of mothers. A higher pup weight (10, 9.7, 10.4, 10.7* and 11.0* g on day 4 resp) and litter weight (120, 124.8, 132.3*, 136.9* and 137.1* g resp, *$p<0.05$-0.001) was noted with no significant effect on implantation loss (24, 28, 23, 24, 22, litters with 2 or less) with the drug or HM(ge). Pre-weaning development was not significantly altered, and slight changes were due to gestational variations.

F1 generation animals: No moralities were observed. Females and males derived from 100 U/kg/bid HM(ge) had lower body weights (males by 7 % and females by 5% at weeks 4-21), also there was an increase in the proportion of dams with a longer (23 day) duration of pregnancy (6, 4, 3, 7 and 16 resp). The age for attaining sexual maturity in F1 females derived from parents treated at 25 or 100 U/kg/bid of X14 was significantly lower compared to controls (33.3, 32.8, 32.6*, 32.4*, and 32.6* days resp). sponsor states that these changes were slight, and not of major toxicological significance. Again with the drug or HM(ge), higher pup weight (10, 9.8, 10.1, 10.4 and 11.5* g on day 4 resp) and litter weight (90.3, 101.6*, 105.1*, 100.2*, and 100.9* g resp, *$p<0.05$-0.001) was noted, with a slightly higher implantation loss (21, 23, 23, 20, 18 litters with 2 or less). No effects on post weaning behavior was observed. A slightly higher incidence of pups with minor kidney changes (mainly increased pelvic dilatation or pale discoloration) in litters derived from females with 100 U/kg/bid HM(ge) was noted.

In conclusion, In a segment III study in rats, old process X14 was given subcutaneously, at doses of 5, 25 and 100 U/k/bid, or HM(ge) 100 U/kg/bid from days 6 of pregnancy to day 21 of lactation period (from implantation to weaning). At 25-100 U/kg/bid of X14, the drug caused 3 deaths/group (vs there were 8 deaths with 100 U/kg/bid of HM(ge), all due to hypoglycemia. Reduction in plasma glucose was noted with X14 and human insulin, and at low-mid doses the blood glucose recovery was seen by 4 hrs. However, at high dose (100 U/kg/bid), the animals showed persistent hypoglycemia with X14 or HM(ge) at 4 hrs. At 100 U/kg/bid of X14, increases in body weight gains (slightly higher increases in body weight were observed with HM(ge)) and

food/water consumptions were noted. In Fo animals, a significant increase in proportion of dams with longer duration of pregnancy (22/23 days) was observed at 25-100 U/kg/bid or human insulin. In F0 offspring, pup weight was slightly increased on day 4 post partum. F_1 rats had lower body weight gain, an increase in proportion of dams with longer duration of pregnancy (23-day), a higher implantation loss in litter, higher pup weight gain on day 4 post partum, and a higher incidence of pups with minor kidney changes. Sponsor states that these effects are due to the maternal pharmacological response to the drug.

Overall Reproductive Toxicology Summary

Following reproductive toxicity studies are summarized here: segment I & II fertility/teratology studies in rats, segment II teratology studies in rabbits, segment III peri-postnatal study in rats.

In a combined segment I/II fertility and embryo-fetal development study in rats, animals were treated with X14 (old process drug) at doses of 5, 25 and 100 U/k/bid, or with HM(ge) 100 U/kg/bid (recombinant human insulin for comparative purposes) subcutaneously. Females were given old process X14 or HM(ge) for 14 days prior to mating, throughout the mating, and from days 0 to day 15 postcoitus. Males were given the drug for 28 days prior to mating, during mating, until their necropsy. One hundred U/kg/bid of X14 and HM(ge) caused 2 and 1 deaths resp, due to hypoglycemia (with associated erosion of stomach wall). Plasma glucose values were decreased with all doses at 1 hr, but took more than 4 hrs to recover (with X14 or human insulin) at 100 U/kg/bid. At 25-100 U/kg/bid, increases in body weight gains (in males by 26-34% and females by 38-58%), and food consumptions (by 10-15%) were observed, along with testicular changes in males (focal seminiferous epithelial atrophy with vacuolation of sertoli cells), and effects on litters were noted (including pre- and post implantation losses, and skeletal and visceral abnormalities, mainly affecting the axial skeleton and eyes). All drug doses reduced blood glucose levels. However the drug had no effects on the male and female fertility or general reproductive performance of animals. The response in general was similar to that seen with HM(ge). Sponsor states that all these effects are due to insulin -induced hypoglycemia, including effects on fetuses.

In a segment II teratology study in rabbits, pregnant animals were treated with old process X14 drug at doses of 0.5, 1.5 and 5 U/k/bid, or with HM(ge) 0.5, 1.5 and 5 U/kg/bid (recombinant human insulin for comparative purposes) subcutaneously from days 6 to day 18 of gestation. Females were sacrificed on day 29 PC and necropsied. At 5 U/kg/bid of X14 or 1.5 U/kg/bid of HM(ge), both drugs caused 1 death each/group, due to hypoglycemia. Dose related reduction in plasma glucose was noted with both X14 and human insulin, and at low-mid doses the blood glucose recovery was seen by 4 hrs. However, at high dose (5 U/kg/bid) the animals showed persistent hypoglycemia with X14 at 4 hrs, but there was a partial recovery with human insulin. At 1.5-5 U/kg/bid of X14, increases in body weight gains (by 59-68% & by up to 44% resp), food

consumptions (by 15-22%), effects on litters, including pre- and post implantation loss, abortions and skeletal abnormalities (mainly affecting the axial skeleton) were observed. The response in general with the drug was similar to that seen with HM(ge). Sponsor states that these teratogenic effects of the drug are due to insulin-induced hypoglycemia, and related to the direct or indirect pharmacological effect of the drug.

In a segment III teratology study in rats, pregnant animals (F_0) were treated with old process X14 at doses of 5, 25 and 100 U/k/bid, or with HM(ge) 100 U/kg/bid (recombinant human insulin for comparative purposes) subcutaneously from days 6 post coitum to day 20 post partum (PP). In a proportion of F_1 females behavioral development and reproductive capacity was examined. At 25-100 U/kg/bid of X14, the drug caused 3 deaths/group (vs there were 8 deaths with 100 U/kg/bid of HM(ge), all due to hypoglycemia. Reduction in plasma glucose was noted with X14 and human insulin, and at low-mid doses the blood glucose recovery was seen by 4 hrs. However, at high dose (50 U/kg/bid), the animals showed persistent hypoglycemia with X14 or HM(ge) at 4 hrs. At 100 U/kg/bid of X14, increases in body weight gains (slightly greater increases in body weight gains were observed with HM(ge)) and food/water consumptions were noted. A significant increase in proportion of dams with longer duration of pregnancy (22/23-day) was observed at 25-100 U/kg/bid. Newborn pups at 100 U/kg/bid of X14 or HM(ge) showed slightly increased weight gain on day 4 PP, which was normalized by weaning. F_1 rats had lower body weight gain, an increase in proportion of dams with longer duration of pregnancy (23-day), a higher implantation loss in litter, higher pup weight gain on day 4 PP, and a higher incidence of pups with minor kidney changes. Sponsor states that these effects are due to the maternal pharmacological response to the drug due to insulin-induced hypoglycemia.

GENETIC TOXICOLOGY:

1) Study Title: Effects of X14 on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

General Information:
Study No: 950375
Volume # and Page #: 26/1
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: February 8, 1996/January 20, 1997
GLP Compliance: Yes, by UK and France compliance
QA- Reports Yes (x) No ( ):
Drug Lot Number: X14-9604
Study Endpoint: Mutagenesis (in vitro, base substitution mutations)

Methodology

Strains Employed: Salmonella typhimurium tester strains TA1535, TA1537, TA98, TA100 (histidine requiring strains which require both biotin as well as histidine for growth), and E. coli tester strain WP2 pKM101, WP2uvrA pKM101 (a tryptophan-requiring strain, in which agents causing base substitution mutations can increase the frequency of Trp revertants). E. coli tester strain WP2 pKM101 and WP2uvrA pKM101 are repair-proficient and repair-deficient plasmid-containing strains resp, the latter is deficient in excition repair at the uvrA locus. The plasmid derivatives TA98, TA100 and E.coli have increased sensitivity to certain mutagens as the pKM101 plasmid codes for error-prone DNA repair systems.

Dose Selection Criteria: The dose selection was based on a previous dose-range study with X14, and no toxicity was observed (study # 930494). Therefore concs of X14 of 312.5-5000 µg/ml were used for both the experiments. The drug was completely soluble at all concs, and no toxicity was observed in both experiments.

Metabolic Activation System: Rat liver microsome S9 fraction.

CONTROLS:
Solvent or Negative Control: 2.5% glycerol.
Positive Controls: Without S9: 2-nitrofluorene (25 µg/ml, for TA98 strain), 4-nitroquinoline1-oxide (NQO 1 µg/ml, for TA100 strain), N-methyl-N-nitrosoguanidine (2.5 & 7.5 µg/ml, for TA98 and E.coli strains), and ICR-191 (1 µg/ml, for TA1537 strain). With S9: 2-aminoanthracene (0.063-0.5 µg/ml, for TA98, TA100, TA1535, TA 1537, and WP2 uvra pKM101 strains).

Exposure Conditions
Methods: Two independent mutation tests were performed. A 'preincubation method' was used. This method has been used for recombinant human insulin previously. In this assay the drug (X14) is incubated with the cells (tester stains) prior to plating, vs the standard plate-incorporation (in which the drugs such as X14 and insulin may cause artifacts due to growth stimulation, both being peptide hormones). The cells were cultured for 10 hrs at 37°C on 0.9% soft agar. The tester strains in the plate were exposed to the vehicle, drug, or positive controls. The cells were incubated for 3 days at 37°C on selective minimal agar, in both the presence and absence of S9 fraction. Colonies were counted manually or electronically.

Concentrations Employed: 312.5-5000 µg/ml.

Analysis
Counting method: Colonies were counted manually (for Expt 1, as low levels of bacterial contamination was observed) or electronically (for Expt 2) using a ——— counter at the end of the study.

Criteria for Positive Genotoxic Results: If the drug induces clear increases in revertant numbers, confirming discrimination between different strains, with a significant

response ($p \leq 0.01$) and a dose related correlation in induced mutant frequency, the drug would be considered mutagenic.

Results:
Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.
Study Outcome: The drug X14 was not mutagenic in any of the tester strains at doses ranging from 312.5-5000 µg/ml in the presence or absence of metabolic activation. However, a significant increase in the number of revertant colonies was observed with the positive controls (with or without S9 mix). In conclusion, the AMES test was negative.

SUMMARY:
Statement: The reviewer concurs with the sponsor that the AMES test was negative for X14.
Labeling Considerations: This negative AMES test is not included in the formal labeling section in this NDA.

2) Study Title: **Effects of X14 on Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes**.

General Information:
Study No: 950373
Volume # and Page #: 26/95
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: February 12, 1996/January 20, 1997
GLP Compliance: Yes, by UK and France compliance
QA- Reports Yes (x) No ( ):
Drug Lot Number: X14-9604
Study Endpoint: Clastogenesis (chromosomal aberrations, in vitro assay)

Methodology
Cells Employed: Cultured peripheral human blood lymphocytes were prepared from the 2 human donors (1 male + 1 female).
Dose Selection Criteria: The highest dose would be the one where 50-80% reduction in mitotic index (MI) would occur, or the highest conc of 5000 µg/ml will be used. Since no clear effects on MI were observed at any dose (5-11% at 5000 µg/ml with or without S9), and the vehicle was also not toxic, concs of X14 of 1201-5000 µg/ml were used for both the experiments.

Metabolic Activation System: Rat liver microsome S9 fraction.

CONTROLS:

Solvent or Negative Control: 2.5% glycerol, and untreated culture medium
Positive Controls: Without S9: 4-nitroquinoline1-oxide (NQO 1.25-5 μg/ml). With S9: cyclophosphamide (12.5-25 μg/ml).

Exposure Conditions
Methods: Two experiments were carried out. Replicate human lymphocyte cell cultures were exposed to various concs of X14. The treatment time was continuous for ≈ 20 hrs and 44 hrs (with 20 and 44 hrs harvest time) without metabolic activation, and 3 hrs (of pulse treatment, with 20 and 44 hrs harvest time), in the absence of metabolic activation, in Expt 1 and 2 resp. Positive and negative controls were similarly treated. This method has been used for recombinant human insulin previously.

Concentrations Employed: 1201-5000 μg/ml.

Analysis
Counting method:
At the end of the study, at least 100 metaphases/culture were examined for structural aberrations including or excluding gaps, and with polyploid, endoreduplicated or hyperdiploid cells. Mitotic index (MI) was determined by examining 1000 cells/culture.

Criteria for Positive Genotoxic Results: If percentage of cells with aberrations (excluding gaps) are increased, with one or more doses demonstrating a statistical significant increase, in relation to the vehicle or negative control (or from a normal range), and the test was confirmed in the second expt, the drug would be considered positive.

Results:
Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.
Study Outcome: The drug X14 did not show any increase in structural chromosome aberrations, at doses ranging from 1200-5000 μg/ml in the presence or absence of metabolic activation. The positive controls (with or without S9 mix) showed a significant increase in aberrations . In conclusion, the drug was not clastogenic in this assay.

SUMMARY:
Statement: The reviewer concurs with the sponsor that the chromosome aberration test in cultured human peripheral blood lymphocytes was negative for X14.
Labeling Considerations: This negative test is not included in the formal labeling section in this NDA.

3) Study Title: **Effects of X14 on Mouse Lymphoma forward Gene mutation assay.**

General Information:

Study No: 950376
Volume # and Page #: 26/329
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: February 8, 1996/January 15, 1997
GLP Compliance: Yes, by UK and France compliance
QA- Reports Yes (x) No ( ):
Drug Lot Number: X149604, recombinant human insulin (RHI) # H02606
Study Endpoint: Mutagenesis (in vitro assay)

Methodology
Cells Employed: Mouse lymphoma L5178Y TK $^{+/-}$ cell line, heterozygous at the tyrosine kinase locus, were obtained from [redacted] LY 5178Y are more sensitive than CHO or V79 cell lines, when assessing the mammalian cell gene mutation assays.
Dose Selection Criteria: The preliminary study showed no toxicity of the drug in 2.5% v/v glycerol (in 2.7 ml volume) vehicle, therefore highest doses of 5000 µg/ml were selected for this study for X14 or RHI . Dose ranges were 312-5000 µg/ml.

Metabolic Activation System: Rat liver microsome S9 fraction.

CONTROLS:
Solvent or Negative Control: 2.5% glycerol diluted in the culture medium (RPMI)
Positive Controls: Without S9: 4-nitroquinoline1-oxide (NQO 0.05-0.1 µg/ml). With S9: benzo(a)pyrene (BP 2-3 µg/ml).

Exposure Conditions
Methods: Cells (At least 10^7) with or without S9 activation mixture were incubated for 3 hrs with the indicated conc of the drug (X14), along with positive and negative controls. Cultures were maintained for a period of 2 days, during which the TK mutation would be expressed. At the end of the experiment, the mutant frequency, 5-trifluorothymidine resistance (the large and small colonies were scored here), cell survival and cell viability (8-11 days) was determined .

This mutation assay works by placing treated cells under selective pressure, so only mutant cells are able to survive. For example resistance to 6-thioguanine (6GT) results from lack of hypoxanthine phosphoribosyl transferase (HPRT) activity and resistance to 5-trifluorothymidine (TFT) form lack of thymidine kinase (TK). Thus HPRT and TK are unable to use the toxic analogs 6GT and TFT and survive their presence, and detect different range of mutation types. Hprt is a large gene, detects point mutations and changes of 30-40 kilobases of DNA. In contrast the tk locus (11-13 kilobases) is autosomal, and 2 types of TFT-resistant colonies (large and small growing colonies) could be selected here. Large colonies tend to represent events within the gene (base pair substitution and deletions), whereas small colony mutants often involve large genetic changes frequently visible as chromosome aberrations. Thus in this system,

gene mutations within the tk gene and chromosomal events involving the gene may be detected.

Concentrations Employed: 312-5000 µg/ml.

Analysis
Determination of mutant frequency (MF) and viability: Mutant frequency was expressed as mutants per 10^6 viable cells. Plating efficiencies were determined by comparing the test and control cultures

Criteria for Positive Genotoxic Results: If mutant frequency at one or more doses was significantly greater than the negative control, and there is a dose -dependent linear increase in mutant frequency, and if these effects are reproducible, the drug would be considered positive.

Results:
Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.
Study Outcome: Two experiments were carried out. In the first expt 312-5000 µg/ml were used. In the second expt 1000-5000 µg/ml of the drug were used. In this assay the number of wells with small and large colonies were examined. The drug X14 (or human recombinant insulin) did not cause any toxicity at doses ranging from 312-5000 µg/ml in the presence or absence of metabolic activation, Also both (X14 or human recombinant insulin) showed no significant differences in mutant frequencies between vehicle control and treated samples in the mouse lymphoma assay. The positive controls (with or without S9 mix) showed a significant increase in mutant frequencies . The proportion of small colony mutants in the vehicle controls were 53 and 57% resp in the absence and presence of S9 (with the positive controls these were in the range of 61-71%). In conclusion, the drug (X14) or recombinant human insulin (RHI) did not induce mutations at tk locus in the mouse lymphoma cells.

SUMMARY:
Statement: The reviewer concurs with the sponsor that mouse lymphoma (with mutation at TK locus) test was negative for X14.
Labeling Considerations: This negative test is not included in the formal labeling section in this NDA.

4) Study Title: **Effects of X14 on in Vivo Micronuclei in Mice.**

General Information:
Study No: 950372
Volume # and Page #: 26/182
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: February 12, 1996/January 20, 1997

GLP Compliance: Yes, by European standard (UK and France compliance)
QA- Reports Yes (x) No ( ):
Drug Lot Number: X14-C96014
Study Endpoint: Clastogenesis (this assay determines the chromosome damaging activity in vivo, erythroblasts in the bone marrow undergoing their last chromosome replication are the target cells here)

Methodology
Test strain and Cells Employed: Out-bred CD-1 mice, males 26-30 g, females 20-24 g.
Dose Selection Criteria: The highest dose selected was 2000 U/kg/day, which corresponds to ≈2000 fold the therapeutic human dose (in man normally would be 1 U/kg/day), 500 and 1000 U/kg/day were chosen as low-mid doses for the study. —— of stock solution was used, and diluted with the X14 medium (containing glycerol 1.6%, m-cresol 0.172%, phenol 0.15% ——.

CONTROLS:
Solvent or Negative Control: X14 medium (containing glycerol 1.6%, m-cresol 0.172%, phenol 0.15% ——).

Positive Controls: Cyclophosphamide (CP), 40 mg/kg was dissolved in saline.

Exposure Conditions
Methods: Mice (5/sex/group/sacrifice time) were given two subcutaneous injections/day of the drug (of X14) at total doses of 500, 1000 and 2000 U/kg/day, 10 hrs apart (to reflect the human therapeutic regime). A group of mice were similarly treated with the vehicle or CP (positive control). Animals were sacrificed at 24 and 48 hrs after the second administration of the drug, and bone marrow cells were prepared. Cells were stained with filtered Giemsa stain, and total of at least 1000 polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were analyzed, and examined for the presence of micronuclei.

Concentrations Employed: 500, 1000 and 2000 U/kg/day of X14 were given SC to animals.

Analysis
Counting method: Total of at least 1000 polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were analyzed, and examined for the presence of micronuclei.

Criteria for Positive Genotoxic Results: If a statistically significant increase in the frequency of micronucleated PCE was observed, at least at one sampling time compared to the vehicle or historical controls, the drug would be considered positive.

Results:

Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.
Study Outcome: The drug did not induce an increase in micronucleated PCE, or the ratio of PCE to NCE in either male or female mice bone marrow up to doses of 2000 U/kg/day. In contrast cyclophosphamide (CP) induced a significant increase in the MCE in both male and female mice, compared to vehicle control. No signs of toxicity were noted in mice with the drug. Treatment with the X14 in mice caused a significant fall in the blood glucose levels. In conclusion, the drug was not cytogenic in this assay.

SUMMARY:
Statement: The reviewer concurs with the sponsor that the micronucleus bone marrow test.in mice was negative for X14.
Labeling Considerations: This negative test is not included in the formal labeling section in this NDA.

5) Study Title: Effects of X14 on 'Ex Vivo' DNA repair Assay (Unscheduled DNA synthesis) in rat liver hepatocytes.

General Information:
Study No: 950374
Volume # and Page #: 26/254
Conducting Laboratory: ———————————————
Date of Study Initiation/completion: February 13, 1996/January 15, 1997
GLP Compliance: Yes, by European standard (UK and France compliance)
QA- Reports Yes (x) No ( ):
Drug Lot Number: X14-C96014, new process drug was used here
Study Endpoint: Genotoxicity (this assay measures the ability of the drug to cause repairable DNA damage in rat hepatocytes, repair is measured as unscheduled DNA synthesis (UDS) by uptake of radio-label thymidine by ———— in 'ex vivo' assay)

Methodology
Test strain and Cells Employed: Male and female Crl: CD BR rats, 47-55 days old, males 205-246 g, females 176-211 g. Rat liver hepatocytes were isolated from rats.
Dose Selection Criteria: The highest dose selected was 2000 U/kg/day, which corresponds to ≈2000 fold the therapeutic human dose (in man usually 1 U/kg/day), 800 U/kg/day were chosen as lower dose for the study. ——— of stock solution was used, and diluted with the X14 medium (containing glycerol 1.6%, m-cresol 0.172%, phenol 0.15% ———).

CONTROLS:
Solvent or Negative Control: X14 medium (containing glycerol 1.6%, m-cresol 0.172%, phenol 0.15% ———).

Positive Controls: 2-Acetamidofluorene (2-AAF, 75 mg/kg).

Exposure Conditions

Methods: Rats (5/sex/group/sacrifice time) were given two subcutaneous injections/day of the vehicle or drug (of X14) at total doses of 800, and 2000 U/kg/day, 10 hrs apart (to reflect the human therapeutic regime). A group of rats were given 2-AAF (the positive control) by oral gavage. Blood samples were obtained ≈ 1 hr before dosing and at sacrifice. Animals were sacrificed at 2-4 hrs after the second administration of the drug, (positive controls at 12-14 hrs) and hepatocytes were prepared. The hepatocytes are seeded on to coverslips, the cells are labeled with ^{3}H-thymidine (10 μc/ml), after overnight exposure, the cells are fixed with glacial acetic acid : ethanol (1:3 v/v) and then subjected to ———————— procedures. UDS was determined by counting the number of silver grain resulting from ^{3}H-thymidine incorporation in the hepatocyte nucleus. The number of nuclear and mean cytoplasmic grains were recorded and the net grains/nucleus (NNG) were determined. 100 cells were examined/animal. The number of grains present in nucleus minus the mean number of grains in 3 equivalent areas of cytoplasm were determined in 2 slides from each animal

Concentrations Employed: 800, and 2000 U/kg/day of X14 were given SC to animals.

Analysis

Counting method: 100 cells were examined/animal. Nuclear and mean cytoplasmic were recorded and the net grains/nucleus (NNG) were determined.

Criteria for Positive Genotoxic Results: The drug would be considered positive, if it yields the group mean values of more than 5 net grains/nucleus (NNG), with 20% or more of the cells responding, and an increase was seen in both NNG and percentage of cells in repair. The overall NNG values of treated would be compared to controls.

Results:

Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.

Study Outcome: The drug (X14 at 800-2000 U/kg/day) did not produce a group mean NNG value of greater than -1.2, negative control animals produced a group mean of less than zero (with 0.7-1.7% cells in repair). In contrast 2-AAF resulted in NNG values of 7.5 with more than 50% cells in repair. All X14 treated animals showed reductions in blood glucose levels. In conclusion, the drug (X14) was negative in this 'ex vivo' UDS assay.

SUMMARY:

Statement: The reviewer concurs with the sponsor that the ex vivo UDS assay was negative for X14.

Labeling Considerations: This negative test is not included in the formal labeling section in this NDA.

Overall Genetic Toxicology Summary
No mutagenic/genotoxic potential of the drug was seen, when X14 was tested in the following 5 different tests, Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, in vivo micronucleus test in mice, and ex vivo UDS test in rat liver hepatocytes.

SPECIAL TOXICOLOGY STUDIES:

1. Mitogenic effects of X14 vs human insulin and ——— in mammary cancer fibroblasts (MCF-7 cells). Study Number: 970015. This study was conducted by Novo Nordisk A/S, Denmark.

Methods: The aim of this study was to examine the mitogenic potential of X14 relative to human insulin, and ——— to determine if X14 has an excessive or inappropriate mitogenic or growth potential. MCF cells (these cells respond well in this model) were incubated with X14 (Batch # C96014), human insulin (batch # 50167), or — at 10-15 different conc, ranging from 2.5 pM to 2 μM for 24 hrs. The percentage of cells synthesizing DNA (the S-phase fraction) was assessed using ——— The increase in the S-phase fraction reflects mitogenic stimulation of cells leading to an increased fraction of cells producing new DNA as a preparation to cell division. The EC_{50} values were used, to compare the mitogenic potential between different analogs.

Results: Seven independent experiments were conducted. The mitogenic potential of X14 was 0 to 84 times that of human insulin (human insulin was 1, X14's mitogenic potential was between 0 to 84 (in four expts it was 1 to 7, and in 3 expts it was 0-84). In contrast in two experiments — showed the mitogenic potential of 31 and 6 times (in duplicate observations in Expt 1), 8 and 404 times (duplicate observations in Expt 2, note that 404 times was noted only once, which may be an outlier) that of human insulin. Sponsor states that the mitogenic potential of X14 is similar to human insulin, but of — is 31-404 times of human insulin. However, the current studies show that there is a great deal of variability in this test method. The mitogenic potential of — may not be significantly different from human insulin or X14. This study was basically inconclusive, with lot of variability, and with no positive controls to assess the validity of this method.

OVERALL SUMMARY AND EVALUATION:

Introduction: Insulin Aspart (X14) is an analog of human insulin, in which the amino acid, proline, in position β28, has been replaced by aspartic acid. This modification was designed to decrease the part of the molecule responsible for self association. Thus, X14 is monomeric, and after injection, is released quickly from the subcutaneous tissue,

thereby exerting an earlier onset of effect than human insulin, which has to dissociate from dimers to monomers before absorption form subcutis. This might be the underlying mechanism why X14 would be absorbed rapidly and thereby produces fast onset of action without losing other properties of human insulin.

The drug is produced by recombinant DNA technology in the yeast sacharomyces cerevisiae.

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The earlier drug manufacturing process was designated as process A. The later drug manufacturing process was designated as the new drug or process C (this will be used clinically). The main changes from A to C ——

X14 is indicated for the treatment of diabetes mellitus. Dosage is determined by the physician, and depends on the needs of the patient, and should be regularly adjusted according to blood glucose measurements. **The individual insulin requirement is usually between 0.5-1.0 U/kg/day.**

Pharmacology: The primary pharmacology suggest that potency of X14 is similar to human insulin. In ob/ob mice both X14 and human insulin —— decreased moderate hyperglycemia to a similar extent (by 2.4 and 3.2 mmol/l resp). In the mouse blood glucose assay, the potency of X14 was similar (104-105%) to international human insulin standard. The faster action of the drug was shown in pigs, where aspart had a faster action (the X14 disappeared at higher rate of —— hrs, $P<0.05$) after subcutaneous administration than human insulin (—— hrs). Receptor binding studies indicated that the affinity of X14 for the insulin receptor is similar (92%) to that of human insulin, whereas its affinity for the IGF1-receptor in this system is at least 1000 fold lower than that of IGF1.

Safety Pharmacology: Several secondary or safety pharmacology studies (up to 100 U/kg doses in rats and mice, and up to 4 U/kg in pigs) were conducted, no significant differences were observed except the drug caused a blood pressure reduction at 4 U/kg in anesthetized pigs (-15.53 vs 9.9 in controls). In rats, Increased diuresis was noted together with increased urinary excretion of electrolytes at 100 U/kg of X14. At 10-100 U/kg of X14, protein (—— vs 2.9 in controls) and creatinine (—— vs 1.5 in controls) were increased in rats. In mice, single iv administration of X14 prolonged the hexobarbital-induced sleeping time at 10-100 U/kg. The drug slightly reduced spontaneous activity at 100 U/kg of X14 (in 2 of 6 mice, which was also noted in 2 of 6 mice with 100 U/kg HM(ge). All these effects could be prevented by concomitant

administration of glucose, suggesting that these may also be due to hypoglycemia, and these were generally also observed with human insulin (HM(ge).

Pharmacokinetics/Toxicokinetics: The pharmacokinetics (PK/TK) of X14 were assessed using the ——— method, which measures both insulin aspart and native human insulin. Since there was a lack of specific assays for rat/dog C-peptides, the endogenous insulin levels for various species could not be calculated and therefore AUC values were not determined. Also 1-year TK studies were conducted using the old process drug (process A), while 1-month studies were carried out with the new process drug (process C). The PK studies indicate that after a single subcutaneous administration of 1U/kg, half life of the drug was 22 min in rats and 67 min in dogs. After iv administration, it was less than 15 min in rats and dogs (for human insulin it was 14 and 13 min resp). The fast elimination was due to its fast clearance (which was 44 ml/min/kg vs 58 ml/min/kg for human insulin). The increase in half life following sc (vs iv) may be due to prolonged absorption from the injection site. The bioavailability of X14 after sc dosing in rats were 82% (for human insulin it was 90%). In dogs the bioavailability was 100% for both X14 or human insulin. Neither rat or dog exhibited the faster onset of action of X14 or faster sc absorption, because the structural differences in the subcutis (which consist of less lipid) exist in dogs and rats, than in pigs and human (which result in generally faster kinetics after sc absorption). No accumulation of the drug (or for human insulin) was observed in rats or dogs after administration for 8 days. The TK studies showed maximal conc at 1 hr after SC injection, and generally linearity in the plasma conc after 1 hr were observed in both rats and dogs. The drug (X14) was widely distributed throughout the body, the peak tissue levels were attained at 0.5 and 2 hrs after sc dosing in male rats, and decreased with time. By 168 hrs very little was observed. Highest concs were observed in thyroid (321 fold of plasma conc, which is due to release of ^{125}I), aorta (1.6 fold), bladder (1.2 fold), GI contents (3 fold), kidneys (1.3 fold) and vena cava (1.6 fold of plasma conc). Similar pattern of distribution were noted in healthy pregnant rats at 19 days of gestation. The radioactivity in fetuses was 6-10% of plasma, which decreased by 24 hrs. The placental transfer of the drug (X14) was small, but the drug was incorporated into the milk of lactating animals (the radioactivity in milk was 2.9, 42.3, 73 and 1.8 pmol/ml at 0.5, 2, 4, and 24 hrs resp). The ratio of AUC (0-24 hrs) of milk to plasma was 30:1. The mean plasma protein binding of X14 was low in rats (0-29%), dogs (0-2%), pigs (11-23%), and man (0-10%). Thus, highest protein binding of 10% was observed in human plasma, with no sex differences. The drug is rapidly and extensively metabolized to mostly smaller peptides and also possibly to ^{125}Iodide. The rapid metabolism was confirmed in plasma, urine, feces and tissue samples of rat. There were no sex differences in metabolism. Most of the drug is excreted in the urine (77% and 87% of the dose resp in male and female rats), while 10.4% and 8.7% was excreted in feces, and the rest in organs and carcass, 17.3 and 6.7 % resp.

Safety Evaluation: This is based on toxicity studies in animals, X14 has been evaluated in the repeat dose toxicity studies of up to 1-year in rats and dogs. Studies of reproductive and developmental toxicity and specific studies regarding mitogenic effects

of the drug vs regular human insulin have been performed. The major toxicity noted in the 1-year studies of rats and dogs was hypoglycemia, which is the pharmacologic or therapeutic action of the drug. Deaths were noted in these studies even at the lowest doses in rats (10 U/kg/day, or 60 U/m^2/day) and at highest doses in dogs (2 U/kg/day, or 40 U/m^2/day), due to the prolonged administration of the drug. The usual clinical human dose is 0.5-1.0 U/kg/day (or 19-37 U/m^2/day). However, in both these studies the actions of regular human insulin and the current drug (at highest doses of 200 and 2 U/kg/day examined in rats and dogs resp) were generally similar, i.e regular human insulin also caused similar number of deaths in animals. These severe or fatal hypoglycemic actions were seen after several days to few weeks of repeated administration of the drug, and may be due to gradual depletion of visceral glycogen stores etc. X14 has similar affinity for the insulin receptor (92%), as human insulin (100%), but affinity of X14 (and insulin) for the IGF-1 receptor is at least 1000 fold lower than that of IGF-1. The in vitro mitogenicity studies of X14 vs regular human insulin in mammary cancer fibroblasts (MCF-7 cells) were inconclusive. In acute toxicity studies the single sc doses of 4000 U/kg (24000 U//m^2/day) in rats and 64 U/kg in dogs (1280 U//m^2/day) were not fatal, as no mortalities were observed. The drug does not accumulate with time, as after 1-year the plasma concs in rats did not increase significantly, and in dogs they were slightly higher. No AUC values could be determined in above studies due to the lack of a specific assay for rat/dog C-peptides, and the available ——— measured both insulin aspart and native human insulin.

The drug (X14) induced dose related increases in antibody titers, in both 1-month rat and dog toxicity studies, which were also observed during the 1-month drug free recovery period in rats. However in 1-year studies in both species, a very few animals had these antibodies with low titers. These were also not neutralizing antibodies, as antibodies had no effect on the reduction of glucose in animals and hypoglycemic deaths were still observed with long term treatment (1-year) even at low doses of the drug.

As indicated earlier, a change in manufacturing process (from process A to C) was introduced in the drug development ——— The process C drug will be used clinically. Although 1-year toxicity studies in rats and dogs were conducted with the earlier drug (process A), 1-month rat toxicity study compared the effects of the old (process A, at a high dose), with the new drug (process C). Generally no differences in toxicities were observed with the change in drug manufacturing process in the 1-month rat toxicity study. In dogs, 1-month toxicity study was conducted only with the new drug. Acute toxicity studies and safety/secondary pharmacology studies were also conducted to compare the effects of the old process drug with new drug. Generally the differences in toxicity, between the two drug processes were not significantly different.

Other Clinically Relevant Issues:
Carcinogenicity: **Effects of X14 on promotion of mammary gland tumors:**
Regarding the carcinogenic potential of X14, the standard 2-year bioassay to determine

the carcinogenicy of the drug in rats and/or mice have not been performed. However, the incidence of mammary gland tumors was examined in two 1-year toxicity studies in rats in this NDA, with a high dose insulin comparator arm. This is because X14 (and insulin) can act as a growth factor, and like insulin, it has a growth promoting potential. One of these studies was an exploratory study in female rats only (draft and not the final QA report was provided for this study).

In the first exploratory 1-year toxicity study in rats (or study 'A'), the effects of ——— were also examined with X14 and human insulin (actrapid). Unlike X14, the ——— has higher affinity for the insulin receptor (205%), compared to both human insulin (100%) or X14 (92%). ——— also has higher affinity for the IGF-1 receptor (0.2%), compared to both, human insulin (0.03%) or X14 (0.05%, affinity of IGF-1 to IGF-1 receptor was 100%), thus affinity of —— for the IGF-1 receptor is 4-fold higher than that of X14. In this study A (n=20/group, **all doses of the drug were given at 200 U/kg/day**), the number of rats that had benign tumors were 4/20 (controls), 7/18 (X14), 11/17 —— and 8/17 (actrapid). The malignant tumors in these groups were 1/20, 4/18, 3/17 and 3/17 resp. Combined analysis (by Peto et al) of total benign + fatal adenomas indicated that ——— was associated with higher incidence of tumors ($p<0.01$), actrapid was also positive compared to control ($p<0.05$). The total number of benign mammary tumors (multiple tumors per rat) were 6/20 (controls), 11/18 (x14), 26/17 ——and 11/17 (actrapid). Thus, in study A, ——— at 32 times the maximum human dose, based on body surface area) was associated with higher incidence of mammary tumors than vehicle control, but this study indicated that the tumorigenic potential of X14 was no greater than endogenous insulin (both at 32 times the maximum human dose, based on body surface area). This exploratory study A also indicated that X14 may have a slightly higher tendency in inducing pituitary gland adenomas than human insulin (3/18 vs none).

In the second regular 1-year toxicity study in rats (or study 'B', GLP and QA certified study), **at 100 U/kg/bid of X14 (or total dose of 200 U/kg/day**, 32 times the maximum human dose, based on body surface area), the incidence of benign mammary gland tumors alone ((8/32* vs 6/32 in controls), or benign with malignant tumors combined (11/32* vs 7/32), was significantly higher with X14 compared to vehicle controls (*$p=0.003$), but similar to regular human insulin compared to controls (6/32 vs 7/32, $p=0.24$). The number of malignant tumors alone, were also higher with X14 at 100 U/kg/bid (4 vs 2 in vehicle controls, but not significantly increased compared to controls, $p=0.090$, with human insulin these were lower than controls, i.e. 1 vs 2 in vehicle controls). **Note that Slight but not significant ($p=0.062$) increases in the incidence of benign + malignant mammary gland tumors was observed with X14 compared to regular human insulin, both given at 100 U/kg/bid.** The pituitary gland adenomas/hyperplasia in study B were not significantly different than those observed with regular human insulin (7/31 vs 8/32).

Note that above two 1-year studies A and B were not identical, and there were following differences in these two studies: 1) The doses were once a day in study A (200 mg/kg/day), and twice a day in study B (100 mg/kg/bid, total dose 200 mg/kg/day). 2) The final X14 doses were different in study A (200 mg/kg/day) vs study B (75 mg/kg/day). 3) At termination (1-year), the animal survival in study A was 85% (even though these animals were receiving 200 U/kg/day throughout the study) vs 44% in study B (even when the doses were reduced from 200 to 100 U/kg/day in week 25, and then to 75 U/kg/day in week 38), no explanation was provided. 4) Study A was a non-QA draft report (we do not know the validity of the data), whereas study B was a QA report. 4) In a regular 2-year carcinogenicity study in animals, data on tumors are usually analyzed by a statistician in the agency, these data were not subjected to that. 5) Both 1-year studies were conducted in mammary prone Sprague-Dawley CD rats. Therefore, these discrepancies may explain the final differences or outcome, in the above two 1-year studies in rats. Overall, the significant increases in mammary tumors occurred with X14 (compared to vehicle controls) at relative high multiples of human doses, and suggest that these may not likely be relevant to clinical use.

No carcinogenicty studies with X14 have been conducted, sponsor states that like insulin, this drug is a large protein, and insulin has been in clinical use for 50 years, with no epidemiological link with cancer in man. Also they state that Sprague Dawley CD rats are prone to spontaneously developing mammary gland neoplasms at 1 year of age, and given the growth promoting effects of the drug (or insulin), it is not surprising that the marginal increase in these tumors is noted, but no other neoplastic lesions were found in the regular 1-year rat/dog studies. Studies on the mutagenic potential of X14 and regular human insulin do not show any significant differences between the two products. The affinity of X14 for the insulin receptor is similar to that of human insulin (92% with X14, vs 100% with human insulin), and its affinity for the IGF-1 receptor is also not significantly different from human insulin (0.05% with X14, 0.03% with human insulin, vs 100 % with IGF).

Thus, study B in rats indicates (QA study) a slightly higher potential of X14 in promoting mammary gland tumors compared to insulin (p=0.062). Study A indicates that tumorogenic potential of X14 was no greater than human insulin, but [redacted] clearly has this potential as well, both of these effects are observed at 32-times the human dose. As indicated earlier that only limited conclusions can be drawn from the comparisons of these two studies. Based on 1-year QA toxicity study, **the current studies in rats suggest that tumorigenic potential of X14 may be higher than insulin, but less than [redacted] Therefore, further studies [redacted] may be warranted to clearly establish the role of X14 in promoting mammary gland tumors in animals.**

Mitogenicity: The in vitro mitogenicity studies indicated that mitogenic potential of [redacted] (6-404 times that of human insulin) was not significantly different from regular human

insulin (human insulin was 1) or X14 (0-84 times that of human insulin), and are therefore inconclusive.

Mutagenicity: X14 was not mutagenic or genotoxic when tested in the following 5 different tests, Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, ex vivo UDS test in rat liver hepatocytes, and in vivo micronucleus test in mice.

Fertility/Pregnancy/Teratogenic Effects: There is no information on teratogenic effects of X14 in humans. Reproduction and teratology studies have been conducted with X14 (and with human insulin for comparative purposes) in rats by subcutaneous injections at total daily doses of up to 200 U/kg/day (i.e. 1200 units/m^2/day or 32 times the maximum recommended human dose of 1 U/kg/day, based on body surface area), and in pregnant rabbits at total daily sc doses up to 10 U/kg/day (i.e. 120 units/m^2/day or 3-times the human dose, based on body surface area). These studies have not revealed any direct adverse effects on fertility, mating performance, or reproductive capacity of animals, and the effects were not generally different from those with regular human insulin at similar doses. However, in rats and rabbits (at 32, and 3 times the human dose resp), both X14 and regular human insulin caused pre- and post-implantations losses in litters, and visceral/skeletal abnormalities in fetuses. This toxicity may be due to insulin-induced maternal hypoglycemia.

The proposed application of X14 for the treatment of diabetes mellitus is recommended for approval.

LABELING

The indicated labeling of X14 generally conforms to the format under CFR 21, 201.50 to 201.57, dated April 1, 1996. However, following changes are requested.

Carcinogenicity, Mutagenicity, Impairment of Fertility

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Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of ——— In 52 week studies with rats dosed subcutaneously with ———