**MONKEY CHRONIC TOXICOLOGY STUDY**

**1-Year Oral Toxicity Study of SK&F 101468 in Cynomolgus Monkeys** (report TP0015/SKF-101468/1)

Note: This was the second 1 yr study initiated. The previous study at was cancelled following the early death of several animals (investigated by DSI with the conclusion that infections were involved but that the drug could not be dissociated from the observed toxicity).

DOSE SELECTION

In a 26-34 week oral toxicity study in cynomolgus monkeys a dose of 30 mg/kg/d (increased from 15 mg/kg/d after week 8) required that 2 males be killed because of their poor condition, one because of self-mutilation and the other due to a severe clinical response to dosing (hyperactivity, agitation and dyskinesia). Clinical signs were pronounced, with the males @ 30mg/kg/d showing minimal weight gain, hyperactivity, stereotypy and formication. 15 mg/kg/d was selected as the HD.

METHODS

Species:
Cynomolgus monkey (wild) 4/s/group from 3 suppliers: . Mean Wts for groups at study initiation: M 3.4-3.75 kg, F 2.7-2.9 kg.

Treatment:
0, 1.5, 5, 15 mg/kg/d base equivalent HCl salt in water by gavage.

Observations:
Clinical signs (daily), body weight (weekly), ECG (6 and 12 months), hematology/serum chem/urinalysis (3,6,9,12 months), ophthalmology (0 and 12 months), prolactin (12 months), PK (days 1, 36, 366), pathology (necropsy).

RESULTS

Mortality:
None

Clinical signs:
Stereotypy (stereotyped locomotion, excessive grooming, formication) occurred mainly days 5-9 and only at the HD (6/8).

Salivation only occurred in treated groups but did not show a clear dose-response. Emesis occurred in all groups, probably as a preconditioned response (Table 40).

Body weight:
Figs 33 and 34: Only HD M shoed a reduced body weight gain (24% vs 38% for controls).

ECG:
No drug effect noted

Ophthalmology:
1 con and 1 LD F had retinal atrophy, but early signs had been noted for the treated F prior to study initiation.

Hematology:
Variable abnormalities were shown in HD groups for lymphocytes (HD F +46%), monocytes (HD F + 117%) and eosinophils (HD F +123%; HD M -95%). No clear dose response was seen. Sponsor considers these incidental.

Hemostasis:
No drug effect on prothrombin time or activated partial thromboplastin time.

Serum chemistry:
No drug effect noted.

Urinalysis:
No drug effect noted.

Endocrinology:
Mean prolactin levels were decreased with some dose relationship (HD M -89%, HD F -66%), but variability between animals was high so that only the HD M was significantly different (Fig 35).

Pathology:
Macroscopic lesions were not observed. Relative adrenal weights were increased in both HD F and M (40-50%). HD M also had increased testes and epididymis mass (50-60%) and an apparent increase in brain weight (24%) which was not seen in F. HD F showed increased ovary weight (120%) which was ascribed to cysts that occur spontaneously in this species.

No clear drug-related microscopic pathology was noted. Ovarian cysts may have significantly contributed to increased ovarian weight.

Brain sections indicated minimal mineralization in the caudate-putamen of 2/4 HD M, and meninges (1/4 MD F), and some inflammatory cell infiltration in the meninges (1/4 HD M) and choroid plexus (1/4 LD M). These effects were not observed in controls.

Toxicokinetics:
There was high variability within groups for days 1, 36 and 366 (2-7 fold range of values for Cmax and AUC) (Tables ) which does not allow assessment of linearity, although there was a clear increase with dose. The apparent half-life of SKF 101468 after 15 mg/ml was 399, 531 and 408 min on days 1, 36 and 366 respectively. Tmax was also variable at all dosages on each day (30-240 min).
There was minimal accumulation indicated by the 0 and 24 hr determinations, [check if consistent vs T1/2 above and ADME section].

The primary metabolite in monkey, SKF 104557 showed an increase in plasma concentration and AUC (0-T) with dose of SKF 101468, although data were variable. [note PK study has AUC ratio of 337 for 1.5 mg day 1- check other data- sponsor considers tocicokinetic AUC error due to low levels]. The ratio for metabolite: parent drug tended to decrease as drug dose increased, suggesting a saturable metabolic process.

| | 1.5 mg/kg | | | 5 mg/kg | | | 15 mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | 36 | 366 | 1 | 36 | 366 | 1 | 3 | 366 |
| Mean data for SKF 101468 | | | | | | | | | |
| Cmax ng/ml | 2 | 13 | 5.6 | 20 | 27.5 | 25 | 195 | 108 | 184 |
| AUC ng.hr /ml | 227 | 1537 | 955 | 3250 | 4715 | 4157 | 34420 | 18190 | 30660 |
| Mean data for SKF 104557 | | | | | | | | | |
| Cmax ng/ml | 56 | 294 | 157 | 480 | 791 | 553 | 2630 | 2630 | 3140 |
| AUC ng.hr /ml | 18030 | 58420 | 637660 | 141400 | 214500 | 173000 | 789300 | 980700 | 819500 |
| Mean Ratios AUC (0-T) 104557:101468 | | | | | | | | | |
| | 95 | 45 | 86 | 52 | 50 | 44 | 23 | 59 | 37 |

EVALUATION

The HD, 15 mg/kg/d produced a reduction of body weight gain and clinical signs primarily within the initial 9 days. Minimal pathology was observed, with changes in testes and ovaries likely to be due to prolactin inhibition. Unfortunately, self-mutilation occurred in isolated cases at higher doses (30 mg/kg/d) which led to the selection of the lower dose (15 mg/kg/d) as an upper limit.

The use of single oral doses compared to t.i.d.. dosing led to a difference in pharmacokinetic profile, with minimal accumulation. Nevertheless, 15 mg/kg/d gave a ROP Cmax 5-fold higher than human Cmax and AUC 0.92 times human daily exposure. Cmax and AUC exposures for SKF 104557 were 80-fold and 19-fold higher than human, while SKF 89124 was not analyzed.

The study indicates that primary toxicities observed with chronic use are likely to be due to DA receptor agonism.



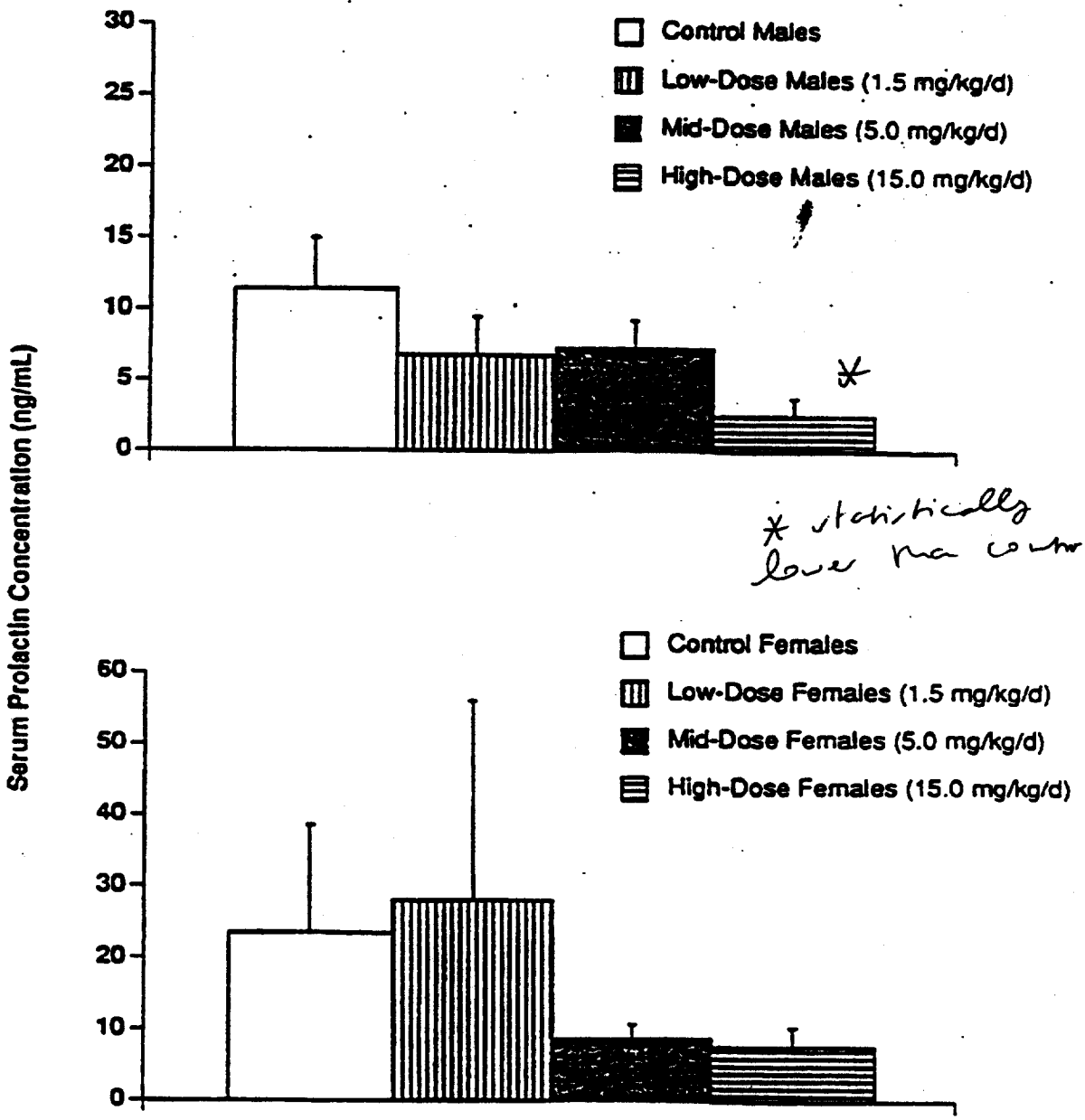
APPEARS THIS WAY
ON ORIGINAL

48a

Figure 3

**1-Year Oral Toxicity Study of SK&F 101468-A in Cynomolgus Monkeys**

**Mean Serum Prolactin Concentration in Male and Female Cynomolgus Monkeys after approximately 1-Year of Treatment**




* statistically lower than control

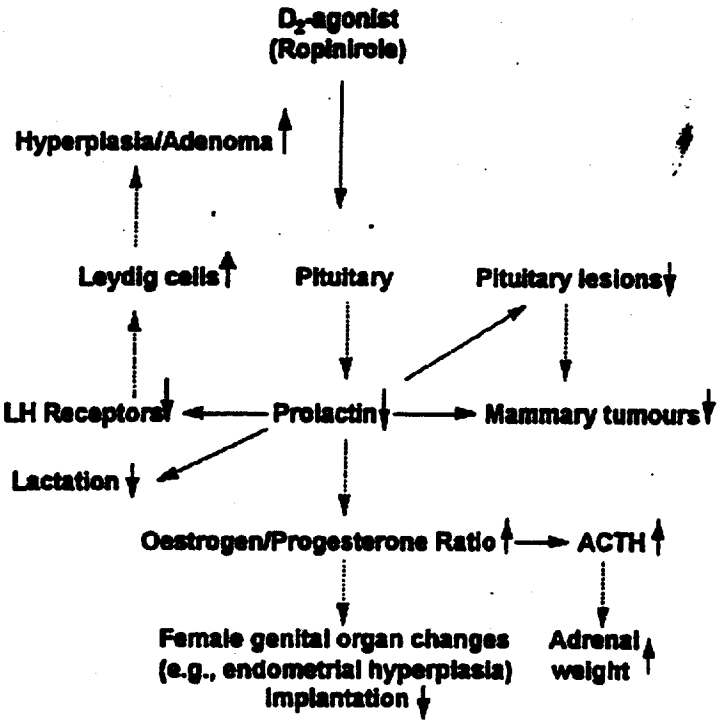
Fig 35

Toxicity studies in the mouse up to 3 months (≤250 mg/kg) [9,10,11] and the mouse carcinogenicity study (≤50 mg/kg) [25] did not reveal macroscopic or microscopic changes that could be attributed to prolactin inhibition.

J 6

~~Figure 2.E.2.1~~

**Effects of $D_2$ Agonist-Mediated Prolactin Lowering in the Rat**




A 30-day study in the rat employing dosages up to 250 mg/kg revealed increased ovarian weight associated with enlarged and reddened ovaries [14]. Microscopically, a dose-related increase in the number of corpora lutea was detected at dosages ≥10 mg/kg. There was also an increase in adrenal weight in females which was associated with a widening of the two inner zones of the adrenal cortex (zonae fasciculata and reticularis). In a subsequent 30-day study, it was demonstrated that the no-effect dose for the phenomenon of persistent corpora lutea was between 5 and 10 mg/kg [15]. The phenomenon of persistent corpora lutea is attributed to the inhibitory effect of ropinirole on prolactin secretion. Besides its luteotrophic role, prolactin also acts as a structural luteolysin in the rat and is responsible for the final structural demise of the corpora

Fig J6

## GENOTOXICITY

**Ames Metabolic Activation Test to Assess the Potential Mutagenic Effects of SK&F 101468 and Dopamine Hydrochloride (report TW008BA)**

DOSE SELECTION

From dose ranging studies. 50-5000 µg/plate.

METHODS

Number of His-revertant colonies determined using strains defective in DNA repair (uvr B-) and lipopolysaccharide cell wall barrier (rfa-).

RESULTS

Table Mean # revertants

| | TA 1535 S9 - | + | TA 1537 S9 - | + | TA 1538 S9 - | + | TA 98 S9 - | + | TA 100 S9 - | + |
|---|---|---|---|---|---|---|---|---|---|---|
| Test 1 | | | | | | | | | | |
| ROP 5000 | 12 | 11 | 14 | 14 | 8 | 10 | 24 | 20 | 110 | 107 |
| solvent | 11 | 13 | 11 | 15 | 10 | 9 | 21 | 28 | 95 | 128 |
| +ve ctrl | 134 | 130 | X | 46 | 78 | 78 | 134 | 122 | 480 | 370 |
| Test 2 | | | | | | | | | | |
| ROP 5000 | 14 | 14 | 7 | 11 | 8 | 8 | 26 | 25 | 91 | 98 |
| solvent | 16 | 19 | 11 | 12 | 8 | 11 | 23 | 28 | 99 | 121 |
| +ve ctrl | 661 | 98 | X | 78 | 75 | 217 | 151 | 250 | 362 | 421 |

X= too high to count
Standard deviations were acceptable. No record was available re cytotoxicity.
Positive controls: Table 41

EVALUATION

No evidence of mutagenicity was obtained.

**Mutation Testing with E. coli WP2 uvrA and WP2 uvrA pKM101 Agar Plate Assay (report TF-1003/SKF-101468/1)**

DOSE SELECTION

312.5-5000 µg/plate from dose range study.

METHODS

Triplicate test plates were used.

RESULTS

No clear cytotoxicity was observed.

Table of Mean # revertants

| | WP2 uvrA S9 - | WP2 uvrA S9 + | WP2 uvrA pKM101 S9 - | WP2 uvrA pKM101 S9 + |
|---|---|---|---|---|
| Test 1 | | | | |
| ROP 5000 | 43 | 77 | 45 | 139 |
| solvent | 52 | 69 | 50 | 159 |
| +ve ctrl | 572 mito | 393 acri | 510 meth | 390 acri |
| Test 2 | | | | |
| ROP 5000 | 45 | 81 | 114 | 119 |
| solvent | 50 | 59 | 110 | 137 |
| +ve ctrl | 510 | 476 | 1096 | 484 |

acri = acriflavine
mito = mitomycin
meth = methylmethane sulphonate

EVALUATION

No evidence of mutagenicity

**Study to determine the ability of SK&F 10146-A and dopamine hydrochloride to induce mutations to 6-thioguanine resistance in mouse lymphoma L5178Y cells using a fluctuation assay (report TW010BA)**

DOSE SELECTION/TREATMENT

Cells were treated for 2 hours, which is below the OECD recommended incubation time of 3-6 hrs, (+/- S9 for each dose in duplicate) then washed. From recovery and growth during the 7-day expression period in flasks, and colony formation in survival

study plates, doses were selected for 6-thioguanine resistance (HGPRT-). No cytotoxicity was noted in expt 1 so the 4 highest doses were used in expt 2.

Expt 1: 0, 158, 500, 1580, 5000 µg/ml
Expt 2: 0, 2000, 3000, 4000, 5000 µg/ml

RESULTS

See Tables 42-45.

In the absence of S-9 no increase in mutation frequency was noted in either experiment. In the second experiment cytotoxicity was noted above 2000 µg/ml, with 5000 µg/ml having only 16 % relative survival. Survival was 94% in experiment 1.

In the presence of S-9 a significant increase in mutagenicity was observed at 5000 µg/ml in both studies (2-3 fold), with relative survival of 90-120%. Doses of 1580 or 2000 µg/ml were not mutagenic, while in the second experiment the intermediate doses (3000 and 4000 µg/ml) were significantly increased or close to significance.

EVALUATION

ROP should be considered as a weak mutagen in the mouse lymphoma test. The compound did not meet all the criteria for positive mutagenicity defined by the sponsor (reproducible dose-response relationship with no overlap in 95% confidence limit ranges for significance vs control), but significant findings at the HD in both assays and reduced effects at 3000-4000 µg/ml in the second assay suggest a dose-related low-potency action between 2000-5000 µg/ml. Additionally, the treatment duration was 2 hours compared to at least 3 hrs noted in accord OECD guidelines, thus potentially the assay could show greater effects with longer incubation.

**Study to evaluate the chromosome damaging potential of SK&F 101468-A and dopamine hydrochloride by their effects on cultured human lymphocytes using an in vitro cytogenetics assay.** (report TW009BA).

DOSE SELECTION/TREATMENT

An initial study of mitotic index showed ROP had no effect at doses up to 5000 µg/ml +/- S-9, therefore this was the HD.

Expt 1: 625, 1250, 2500, 5000 µg/ml
Expt 2: 1250, 2500, 5000 µg/ml
3 hr treatment duration. Positive controls were methyl methane sulphonate (-S9) and cyclophosphamide (+S9).

Cell cycle analysis using 5-bromo-2-deoxyuridine (counts of cells in their 1st, 2nd or 3rd cell cycle post BudR) indicated that ROP treated cells at 72 hrs were similar to controls at 68 hrs, therefore chrom ab analyses were performed at these times.

Results are noted in Tables 46-51. In the presence of S9 no increase in abnormalities was observable. In the absence of S9 a significant increase in abnormalities was observed for either structural (or structural plus numerical) aberrations, 3-5-fold control values and nearly twice the level of historical upper range. However, this was only seen in the second experiment (in the first experiment a decrease was noted in the equivalent parameters) and lower doses were comparable to controls. Positive controls were active.

EVALUATION

Although aberration values were high at 5000 µg/ml in the absence of S-9 in one experiment, the lack of dose-response relationship and absence of effect in another duplicate experiment suggest an incidental observation. ROP should therefore be considered non-mutagenic in this assay.

In the lymphoma and lymphocyte tests run by Microtest Research Ltd, ROP was dissolved in culture medium, which was used as the negative control solvent. Positive control compounds were prepared in DMSO (concentration not stated), which raises the possibility that enhanced permeability due to DMSO might have enhanced the efficacy of known genotoxic molecules relative to ROP. This is less of a concern with this compound since ROP has a logP of >+2, but the protocol should be modified in future.

**Study to evaluate the potential of SK&F 101468-A to include micronuclei in the polychromatic erythrocytes of CD-1 mice. (report TW011BA)**

DOSE SELECTION

Initial toxicity studies with 200-800 mg/kg indicated mortality @ 600 and 800 mg/kg p.o.. 400 mg/kg was selected as the HD, which produced some mortality in the main study.

METHODS

Species:
CD-1 mice

Treatment:
HD 400 mg/kg p.o. in water (highest non-lethal dose). Lower doses

were 200 and 100 mg/kg. The negative control was water, and the positive cyclophosphamide in water. 15/s/group. 5 animals/s were sacrificed 24, 48 and 72 hrs post-dose.

Observations:
The PCE:NCE ratio was calculated from 200 cells, then counts of PCE continued to 1000 for incidence of micronuclei. NCE were calculated from the incidence during PCE:NCE assay and should be considered less reliable than actual counts of micronucleated PCE.

RESULTS

1M and 1F died @ 400 mg/kg p.o. dose level, which were replaced. The replacement F also died and was not replaced (4F were sacrificed @ 48 hr).

No increase was noted in micronucleated PCEs in the treatment groups examined (400 mg/kg- 24-72 hrs; 200 and 100 mg/kg- 24 hrs). Positive control was active (Table 52).

EVALUATION

The HD was acceptable due to mortality. ROP was not mutagenic in this assay.

53c

TABLE 5

Test 1

Mutability tests with bacterial strains
TA 1535, TA 1537, TA 1538, TA 98 and TA 100

| Strain | Compound | Dose level (µg/plate) | Metabolic activation | Mean revertant colony counts | SD | Individual revertant colony counts |
|---|---|---|---|---|---|---|
| /TA 1535 | ENNG | 5 | - | 134 | 1.0 | 133,135,134 |
| TA 1537 | 9 AC | 80 | - | X | - | X,X,X |
| TA 1538 | NF | 2 | - | 78 | 4.5 | 78,74,83 |
| TA 98 | NF | 1 | - | 134 | 21.8 | 161,110,152 |
| /TA 100 | ENNG | 3 | - | 480 | 47.1 | 494,431,525 |
| /TA 1535 | AA | 2 | + | 130 | 34.6 | 134,94,163 |
| TA 1537 | AA | 2 | + | 46 | 1.5 | 47,46,44 |
| TA 1538 | AA | 0.5 | + | 78 | 10.1 | 84,86,83 |
| TA 98 | AA | 0.5 | + | 122 | 15.9 | 112,113,140 |
| /TA 100 | AA | 0.5 | + | 370 | 48.6 | 358,328,423 |

- \- Absence
- \+ Presence
- SD Standard deviation
- X Too many colonies for accurate counting
- / Data obtained from separate experiments due to poor growth of original cultures
- ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
- 9 AC 9-aminoacridine
- AA 2-aminoanthracene
- NF 2-nitrofluorene

Table 41
+ve controls for AMES test

Study Number: SMT 6/ML

TABLE 1: Percentage relative survival and mutation frequencies for SK&F 101468-A and controls in the absence of S-9.

Experiment 1

| Treatment (ug/ml) | | Relative survival % | Mutation frequency[a] | 95% confidence limits lower | 95% confidence limits upper | Comparison with solvent control |
|---|---|---|---|---|---|---|
| 0 | A+B | 100 | 9.7 | 7.7 | 12.2 | |
| 1.58 | A+B | 63 | NP | | | |
| 5 | A+B | 143 | NP | | | |
| 15.8 | A+B | 143 | NP | | | |
| 50 | A+B | 157 | NP | | | |
| 158 | A+B | 145 | 9.3 | 7.4 | 11.6 | - |
| 500 | A+B | 122 | 13.8 | 11.1 | 17.1 | - |
| 1580 | A+B | 120 | 6.4 | 4.9 | 8.4 | - |
| 5000 | A+B | 94 | 13.9 | 11.2 | 17.3 | - |
| | ↳ plate A + plate B | | | | | |
| Positive control (NQO) | | | | | | |
| 0.1 | A | 16 | 124.2 | 100.0 | 154.2 | * |
| 0.15 | A | 10 | 200.8 | 159.8 | 252.4 | * |

a = 6TG-resistant mutants/$10^6$ viable cells at least 7 days after treatment.
NP = not plated for viability/6TG-resistance.
* = significant increase in mutation frequency by comparison of 95% confidence limits with solvent control.



Table 42

Study Number: SMT 6/ML

TABLE 3: Percentage relative survival and mutation frequencies for SK&F [redacted]-A and controls in the presence of [redacted]

Experiment 1

| Treatment (ug/ml) | | Relative survival % | Mutation frequency[a] | 95% confidence limits lower | 95% confidence limits upper | Comparison with solvent control |
|---|---|---|---|---|---|---|
| 0 | A+B | 100 | 8.6 | 6.2 | 11.8 | |
| 1.58 | A+B | 83 | NP | | | |
| 5 | A+B | 135 | NP | | | |
| 15.8 | A+B | 133 | NP | | | |
| 50 | A+B | 133 | NP | | | |
| 158 | A+B | 130 | 10.8 | 8.0 | 14.6 | - |
| 500 | A+B | 133 | 12.1 | 8.7 | 16.8 | - |
| 1580 | A+B | 141 | 8.3 | 5.9 | 11.7 | - |
| 5000 | A+B | 120 | 16.5 | 12.4 | 22.0 | * |
| Positive control (BP) | | | | | | |
| 2.0 | A | 146 | 39.2 | 27.3 | 56.2 | * |
| 3.0 | A | 87 | 77.9 | 59.1 | 102.6 | * |

a = 6TG-resistant mutants/$10^6$ viable cells at least 7 days after treatment.
NP = not plated for viability/6TG-resistance.
* = signficant increase in mutation frequency by comparison of 95% confidence limits with solvent control.



Table 43

<3.d

Study Number: SMT 6/ML

TABLE 5: Percentage relative survival and mutation frequencies for [redacted] and controls in the absence of S-9.

Experiment 2

| Treatment (ug/ml) | | Relative survival % | Mutation frequency[a] | 95% confidence limits lower | 95% confidence limits upper | Comparison with solvent control |
|---|---|---|---|---|---|---|
| 0 | A+B | 100 | 7.1 | 5.4 | 9.4 | |
| 1000 | A+B | 96 | NP | | | |
| 2000 | A+B | 64 | 8.2 | 6.2 | 10.8 | - |
| 3000 | A+B | 60 | 3.9 | 2.7 | 5.7 | - |
| 4000 | A+B | 22 | 7.1 | 5.3 | 9.4 | - |
| 5000 | A+B | 16 | 4.7 | 3.4 | 6.4 | - |
| **Positive control (NQO)** | | | | | | |
| 0.1 | A | 24 | 47.1 | 36.7 | 60.4 | * |
| 0.15 | A | 11 | 82.6 | 65.8 | 103.8 | * |

a = 6TG-resistant mutants/$10^6$ viable cells at least 7 days after treatment.
NP = not plated for viability/6TG-resistance
* = significant increase in mutation frequency by comparison of 95% confidence limits with solvent control





Table 44

Study Number: SMT 6/ML

TABLE 7: Percentage relative survival and mutation frequencies for [redacted] and controls in the [redacted].

[redacted]

| Treatment (ug/ml) | | Relative survival % | Mutation frequency[a] | 95% confidence limits lower | 95% confidence limits upper | Comparison with solvent control |
|---|---|---|---|---|---|---|
| 0 | A+B | 100 | 3.9 | 2.7 | 5.5 | |
| 1000 | A+B | 106 | NP | | | |
| 2000 | A+B | 93 | 5.2 | 3.9 | 7.0 | - |
| 3000 | A+B | 104 | 7.5 | 5.6 | 10.0 | * |
| 4000 | A+B | 94 | 7.2 | 5.4 | 9.6 | - |
| 5000 | A+B | 89 | 10.0 | 7.8 | 12.8 | * |
| **Positive control (BP)** | | | | | | |
| 2.0 | A | 50 | 86.2 | 68.6 | 108.3 | * |
| 3.0 | A | 76 | 47.3 | 37.5 | 59.6 | * |

a = 6TG-resistant mutants/$10^6$ viable cells at least 7 days after treatment.
NP = not plated for viability/6TG-resistance.
* = significant increase in mutation frequency by comparison of 95% confidence limits with solvent control





Table 45

53f

Metaphase Analysis in vitro

TA1
Exper

summary Form

Study number: SMT 6/HLC

Test chemical: SK&F 101468-A

| Treatment | solvent [redacted] | | | 1250 ug/ml - S-9 | | | 2500 ug/ml - S-9 | | | 5000 ug/ml [redacted] | | | Dopamine HCl 5000 ug/ml - S-9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex of donor | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F |
| No. of cells scored | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 |
| Gaps | 2 | 2 | 4 | 2 | 0 | 2 | 2 | 0 | 2 | 3 | 4 | 7 | 3 | 4 | 7 |
| Chromosome deletions | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 1 | 3 |
| Chromosome exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chromatid deletions | 0 | 3 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 3 | 6 |
| Chromatid exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| Others | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 4 | 4 | 8 |
| Total abs. inc. gaps | 3 | 7 | 10 | 4 | 5 | 9 | 5 | 0 | 5 | 4 | 5 | 9 | 12 | 16 | 28 |
| Total structural & numerical abs. | 1 | 5 | 6 | 2 | 5 | 7 | 3 | 0 | 3 | 1 | 1 | 2 | 9 | 12 | 21 |
| Total structural abs. | 0 | 3 | 3 | 1 | 4 | 5 | 2 | 0 | 2 | 0 | 1 | 1 | 5 | 8 | 13 |
| Mitotic index | 5.2 | 1.5 | 3.4 | 4.2 | 3.1 | 3.7 | 5.9 | 2.7 | 4.3 | 6.7 | 3.9 | 5.3 | 0.9 | 0.9 | 0.9 |

M+F

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % cells with any aberration inc. gaps | 3 | 7 | 5 | 4 | 5 | 4.5 | 4 | 0 | 2 | 3 | 4 | 3.5 | 11 | 10 | 10.5 |
| % cells with structural and numerical abs. | 1 | 5 | 3 | 2 | 5 | 3.5 | 2 | 0 | 1 | 1 | 1 | 1 | 8 | 7 | 7.5 |
| % cells with structural abs. | 0 | 3 | 1.5 | 1 | 4 | 2.5 | 1 | 0 | 0.5 | 0 | 0 | 0 | 4 | 3 | 3.5 |

41

37

28

abs. = aberrations



+ve control without S9 (methylmethane sulfonate) 100µg/ml

Human lymphocyte chrom ab

T.11 4L

JJ

Metaphase Analysis in vitro

TABLE 3
Experiment 1

Summary Form

Study number: SMT 6/HLC

Test chemical: SK&F 101468-A

| Treatment | solvent [redacted] | | | 1250 ug/ml + S-9 | | | 2500 ug/ml + S-9 | | | 5000 ug/ml [redacted] | | | Dopamine HCl 5000 ug/ml + S-9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex of donor | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F |
| No. of cells scored | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 |
| Gaps | 0 | 5 | 5 | 3 | 2 | 5 | 1 | 1 | 2 | 4 | 1 | 5 | 1 | 8 | 9 |
| Chromosome deletions | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 1 | 1 |
| Chromosome exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chromatid deletions | 0 | 2 | 2 | 1 | 0 | 1 | 0 | 2 | 2 | 0 | 1 | 1 | 0 | 1 | 1 |
| Chromatid exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Others | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 3 | 4 | 2 | 0 | 2 |
| Total abs. inc. gaps | 1 | 7 | 8 | 5 | 2 | 7 | 2 | 4 | 6 | 5 | 8 | 13 | 3 | 10 | 13 |
| Total structural & numerical abs. | 1 | 2 | 3 | 2 | 0 | 2 | 1 | 3 | 4 | 1 | 7 | 8 | 2 | 2 | 4 |
| Total structural abs. | 0 | 2 | 2 | 1 | 0 | 1 | 0 | 2 | 2 | 0 | 4 | 4 | 0 | 2 | 2 |
| Mitotic Index | 4.1 | 2.7 | 3.4 | 4.1 | 2.1 | 3.1 | 4.0 | 1.5 | 2.8 | 4.4 | 2.1 | 3.3 | 2.0 | 1.5 | 1.8 |

MH

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % cells with any aberration inc. gaps | 1 | 5 | 3 | 5 | 2 | 3.5 | 2 | 4 | 3 | 5 | 7 | 6 | 3 | 8 | 5.5 |
| % cells with structural and numerical abs. | 1 | 2 | 1.5 | 2 | 0 | 1 | 1 | 3 | 2 | 1 | 6 | 3.5 | 2 | 1 | 1.5 |
| % cells with structural abs. | 0 | 2 | 1 | 1 | 0 | 0.5 | 0 | 2 | 1 | 0 | 3 | 1.5 | 0 | 1 | 0.5 |

51

44

37

abs. = aberrations



+ve control
25 mg/ml
cyclophosphamid

Human lymphocyte



Table 47

TABLE 5
Experiment 2

Metaphase Analysis in vitro — Summary Form

Study number: SMT 6/HLC — Test chemical: SK&F 101468-A

| Treatment | solvent - S-9 | | | 1250 ug/ml - S-9 | | | 2500 ug/ml - S-9 | | | 5000 ug/ml - S-9 | | | Dopamine HCl 5000 ug/ml - S-9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex of donor | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F |
| No. of cells scored | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 |
| Gaps | 1 | 2 | 3 | 6 | 2 | 8 | 2 | 6 | 8 | 0 | 4 | 4 | 8 | 5 | 13 |
| Chromosome deletions | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 5 | 7 | 4 | 11 |
| Chromosome exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chromatid deletions | 0 | 1 | 1 | 2 | 1 | 3 | 0 | 1 | 1 | 2 | 3 | 5 | 1 | 6 | 7 |
| Chromatid exchanges | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| Others | 0 | 3 | 3 | 1 | 0 | 1 | 2 | 3 | 5 | 2 | 3 | 5 | 7 | 2 | 9 |
| Total abs. inc. gaps | 2 | 6 | 8 | 9 | 3 | 12 | 4 | 10 | 14 | 6 | 13 | 19 | 25 | 17 | 42 |
| Total structural & numerical abs. | 1 | 4 | 5 | 3 | 1 | 4 | 2 | 4 | 6 | 6 | 9 | 15 | 17 | 12 | 29 |
| Total structural abs. | 1 | 1 | 2 | 2 | 1 | 3 | 0 | 1 | 1 | 4 | 6 | 10 | 10 | 10 | 20 |
| Mitotic index | 3.9 | 4.3 | 4.1 | 5.0 | 3.9 | 4.5 | 6.9 | 5.0 | 6.0 | 5.2 | 4.7 | 5.0 | 1.6 | 2.2 | 1.9 |
| % cells with any aberration inc. gaps | 2 | 6 | 4 | 8 | 3 | 5.5 | 4 | 7 | 5.5 | 5 | 11 | 8 | 17 | 12 | 14.5 |
| % cells with structural and numerical abs. | 1 | 4 | 2.5 | 3 | 1 | 2 | 2 | 4 | 3 | 5 | 8 | 6.5 | 12 | 8 | 10 |
| % cells with structural abs. | 1 | 1 | 1 | 2 | 1 | 1.5 | 0 | 1 | 0.5 | 3 | 5 | 4 | 6 | 6 | 6 |

abs. = aberrations



Human lymphocyte

Metaphase Analysi — Summary Form

Study number: SMT 6/HLC — Test chemical: SK&F 101468-A

| Treatment | solvent + S-9 | | | 1250 ug/ml + S-9 | | | 2500 ug/ml + S-9 | | | 5000 ug/ml + S-9 | | | Dopamine HCl 5000 ug/ml + S-9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex of donor | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F | M | F | M&F |
| No. of cells scored | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 | 100 | 100 | 200 |
| Gaps | 3 | 0 | 3 | 4 | 0 | 4 | 2 | 2 | 4 | 5 | 3 | 8 | 3 | 4 | 7 |
| Chromosome deletions | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 0 | 1 |
| Chromosome exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chromatid deletions | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 0 | 3 | 2 | 2 | 4 |
| Chromatid exchanges | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Others | 0 | 3 | 3 | 0 | 1 | 1 | 3 | 1 | 4 | 1 | 0 | 1 | 1 | 1 | 2 |
| Total abs. inc. gaps | 4 | 6 | 10 | 4 | 1 | 5 | 5 | 8 | 13 | 10 | 5 | 15 | 7 | 7 | 14 |
| Total structural & numerical abs. | 1 | 6 | 7 | 0 | 1 | 1 | 3 | 6 | 9 | 5 | 2 | 7 | 4 | 3 | 7 |
| Total structural abs. | 1 | 3 | 4 | 0 | 0 | 0 | 0 | 5 | 5 | 4 | 2 | 6 | 3 | 2 | 5 |
| Mitotic index | 4.0 | 3.7 | 3.9 | 5.8 | 2.4 | 4.1 | 5.2 | 4.0 | 4.6 | 5.0 | 4.0 | 4.5 | 2.2 | 3.2 | 2.7 |
| % cells with any aberration inc. gaps | 4 | 5 | 4.5 | 4 | 1 | 2.5 | 5 | 4 | 4.5 | 7 | 5 | 6 | 7 | 6 | 6.5 |
| % cells with structural and numerical abs. | 1 | 5 | 3 | 0 | 1 | 0.5 | 3 | 3 | 3 | 4 | 2 | 3 | 4 | 2 | 3 |
| % cells with structural abs. | 1 | 2 | 1.5 | 0 | 0 | 0 | 0 | 3 | 1.5 | 3 | 2 | 2.5 | 3 | 2 | 2.5 |

abs. = aberrations



Human lymphocyte

Table 45

Study number: SMT 6/HLC

## APPENDIX 4B

### Experiment 2

### STATISTICAL ANALYSIS OF TEST CHEMICAL AND DOPAMINE DATA

CHI-SQUARED VALUES FOR SK&F 101468-A IN THE ABSENCE OF S-9

| | DOSE (UG/ML) | CHI-SQUARED VALUE | SIGNIFICANCE |
|---|---|---|---|
| FOR ABERRATIONS WITH GAPS: | 1250 | 0.45 | NOT SIGNIFICANT |
| | 2500 | 1.14 | NOT SIGNIFICANT |
| | 5000 | 3.7 | NOT SIGNIFICANT |
| DOPAMINE HYDROCHLORIDE | 5000 | 21.78 | P < 0.001 |
| FOR STRUCTURAL AND NUMERICAL ABERRATIONS: | 1250 | 0 | NOT SIGNIFICANT |
| | 2500 | 0 | NOT SIGNIFICANT |
| | 5000 | 4.05 | P < 0.05 |
| DOPAMINE HYDORCHLORIDE | 5000 | 15.56 | P < 0.001 |
| FOR STRUCTURAL ABERRATIONS ONLY: | 1250 | 0 | NOT SIGNIFICANT |
| | 2500 | 0 | NOT SIGNIFICANT |
| | 5000 | 4.08 | P < 0.05 |
| DOPAMINE HYDROCHLORIDE | 5000 | 13.14 | P < 0.001 |

CHI-SQUARED VALUES FOR SK&F 101468-A IN THE PRESENCE OF S-9

| | DOSE (UG/ML) | CHI-SQUARED VALUE | SIGNIFICANCE |
|---|---|---|---|
| FOR ABERRATIONS WITH GAPS: | 1250 | 1.07 | NOT SIGNIFICANT |
| | 2500 | 0.17 | NOT SIGNIFICANT |
| | 5000 | 0.64 | NOT SIGNIFICANT |
| DOPAMINE HYDROCHLORIDE | 5000 | 0.38 | NOT SIGNIFICANT |
| FOR STRUCTURAL AND NUMERICAL ABERRATIONS: | 1250 | 3.13 | NOT SIGNIFICANT |
| | 2500 | 0.06 | NOT SIGNIFICANT |
| | 5000 | 0.07 | NOT SIGNIFICANT |
| DOPAMINE HYDROCHLORIDE | 5000 | 0.07 | NOT SIGNIFICANT |
| FOR STRUCTURAL ABERRATIONS ONLY: | 1250 | 2.25 | NOT SIGNIFICANT |
| | 2500 | 0 | NOT SIGNIFICANT |
| | 5000 | 0.1 | NOT SIGNIFICANT |
| DOPAMINE HYDROCHLORIDE | 5000 | 0 | NOT SIGNIFICANT |



Human lymphocyte

T. 11, 50

Study number: SMT 6/HLC

## APPENDIX 5

## Historical solvent control data for human lymphocyte cultures*

| | | - S-9 | | | + S-9 | | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Defined normal range | Mean | SD | Defined normal range |
| Total aberrations including gaps per 100 cells | M | 3.23 | 3.72 | 0-12 | 2.53 | 2.63 | 0-11 |
| | F | 4.31 | 3.99 | 0-12 | 3.08 | 3.07 | 0-11 |
| Total structural and numerical aberrations per 100 cells | M | 1.85 | 2.38 | 0-8 | 1.54 | 1.56 | 0-7 |
| | F | 2.23 | 1.92 | 0-8 | 1.38 | 1.04 | 0-7 |
| Total structural aberrations per 100 cells | M | 1.23 | 2.13 | 0-6 | 1.00 | 1.35 | 0-5 |
| | F | 1.38 | 1.80 | 0-6 | 1.00 | 1.00 | 0-5 |

* Calculated on the basis of at least 12 of the most recent experiments at 1.12.86.

Human lymphocyte



Table 51

**Oral reproductive sighting studies in rats**

Prolactin is known to be important in the regulation of ovarian function (progesterone production by luteal cells) as well as lactation in the rat.



METHODS
Species:
Wistar rats 7-8 weeks of age, 20 F/group

Treatment:
Daily gavage administration of:
0,5,10,20,30,40,50 or 150 mg/kg/day for the following days (3 separate groups).

i) 15 days prior to mating to E7
ii) E7-E16
iii) E16-lactation day 21 (P21)

RESULTS

i) 50-150 mg/kg/day produced clinical signs (hyperactivity, agitation), transient decreases in maternal body weight and food consumption.
ii) Infertility occurred above 10 mg/kg/day
iii) Abortion occurred above 40 mg/kg/day
iv) Lactation failed above 40 mg/kg/day, and 10-30 mg/kg/day decreased pup weight gain
vi) Administration of 5 mg/kg/day during the prolactin-sensitive period of pregnancy and <30 mg/kg/day during lactation could avoid abortion and post-natal mortality.

These studies were consistent with a luteotrophic action of prolactin up to day E8: administration of ROP can inhibit implantation, or cause abortion when administered following implantation in the rat.

**SK&F 101468-A: Fertility study in the female rat** (report TW025BA)

DOSE SELECTION
A pharmacological result of dopamine D2 receptor activation is hypoprolactinemia. Ovarian progesterone secretion in the rat is prolactin-dependent until about E8.

METHODS

Species:
Wistar rats 7-8 weeks of age, 30/group

Treatment:
Daily gavage administration as shown in Table 53

Observations:
Body weight and clinical signs daily for dams and F1 pups to weaning, and twice weekly for F1 pups to mating. On E21 half the females were sacrificed and ovaries, uterus and fetuses (external malformations noted with 50 % dissected for visceral examination and the remainder for skeletal studies) examined. Pups from the remaining females were assessed for malformations and behavioral development. F1 animals were mated, then the dams and fetuses examined as above.

RESULTS

Maternal Toxicity and Fertility
Body weight. ROP caused a transient, dose-related reduction in dam body weight for several days on initial treatment at 50 or 100 mg/kg/day (1-4%). This effect was smaller when repeated on E9-20.
Mating/fertility. ROP did not change estrous cyle time, the number of females mating or the number of pregnant animals. The number of corpora lutea, implantation sites and live fetuses was increased (Table 54).
One F in the HD group lost a litter.

Fetal toxicity
Few malformations were noted, with no clear dose-dependent effects. No differences were observed in the number of pups/litter (9.6-12.3 across groups).

Postnatal toxicity
There was a dose-dependent decrease in pup weight gain to day P20, with the HD leading to about a 44% decrease compared to control (17.7g vs 31.5 g).
Development was slightly delayed in the two highest dose groups, with about a 1-day lag compared to controls for the time at which pinna unfolding, tooth eruption and eye opening occurred in essentially all animals. A decrease in the proportion of pups able to right themslves was dose-related on postnatal day 5 (78%, 68% and 50% for LD, MD and HD compared to 75% in controls).
F1 animal weights for the two high dose groups remained below control but there was no clear effect on fertility or offspring.

EVALUATION

The doses studied were the maximum doses consistent with pregnancy and lactation during critical periods. ROP did not adversely affect fertility or pregnancy but pups had decreased weight gain and developmental retardation.

**Preliminary Oral teratology study in the rat (report TW020BA)**

DOSE SELECTION

An initial study (TW020BA part A) indicated that 30 mg/kg on E7 and 150 mg/kg on days 8-16 indluced abortion. Therefore, 30 mg/kg/day was investigated as the initial dose, with the dose rising to 150 mg/kg/day on days E9-16, E10-16, or E11-E16, a dose expected to produce some maternal toxicity.

METHODS

Species:
Mated F Wistar rats 10/group

Treatment:
One treatment daily by gavage @ 5 ml/kg. The schedule was as shown below. The animals were sacrificed on day 22.

Days of treatment (post mating)

| Group | 0 | 30 | 150 mg/kg/d ROP |
|---|---|---|---|
| 1 | 7-16 | | |
| 2 | | 7-8 | 9-16 |
| 3 | | 7-9 | 10-16 |
| 4 | | 7-10 | 11-16 |

Observations:
Clinical signs and body weights daily. The uterus (live fetuses and early or late resorptions) and ovaries (corpora lutea) of the dams were studied and fetuses examined for weight, sex and external abnormalities.

RESULTS

Maternal toxicity

Clin signs/mortality. Salivation was noted in most animals after

150 mg/kg doses. Two females treated with 150 mg/kg at day 9 aborted, suggesting prolactin dependence extends to day 10. The groups showed a transient decrease in body weight gain over days 7-12 of 150 mg/kg/day dosing. Subsequent weight gain was similar to controls.

Embryo-Fetal toxicity

Post-implantation loss was elevated in treated groups. Group 2 had 2 complete abortions. Groups 3 and 4, had more late embryonic death (Table 55). Fetal weight was also reduced in group 4 (4g vs 4.6g in control). Malformations were seen in 6/27 litters (Table 55) all of which had digit abnormalities, including fusion, aphalangy (distal phalange/s) or adactyly. Additionally, a group 3 fetus had a cleft palate and a group 4 fetus had cleft palate, reduced jaw and absence of an eyelid. 3 other group 4 fetuses had shortened tails.

EVALUATION

The incidence of 150 mg/kg/d ROP-induced abortion dropped from essentially 100 % to < 10% when administration was on day 8 compared to day 10 of pregnancy. There was a high incidence of fetal malformations, especially an absence or reduction in digits. The sponsor notes that this has not previously been associated with dopaminergic agents, but similar effects can be produced by clamping the uterine vasculature. The mechanism has been postulated to be vasodilation and rupture of vessels in the autopod followed by hypoxia.

**Oral teratology study in the rat** (report TW023BA)

DOSE SELECTION

20 mg/kg/day was selected for E6-E7 since no abortions had been seen at this dose. On days E8-E15 doses up to 150 mg/kg/day were used since this dose caused decreased food consumption and body weight gain. 20 mg/kg/day was expected to be a no-effect dose, and intermediate doses were selected for dose-effect data.

METHODS

Species:
Mated F Wistar rats 25/group

Treatment:
One treatment daily by gavage @ 5 ml/kg. The schedule was 20 mg/kg/d for days E6 and E7, then for days E8 to E15 doses were

20, 60, 90, 120 and 150 mg/kg/d. Controls received water for both periods. The animals were sacrificed on day E21.

Observations:
Clinical signs and body weights daily. Food and water consumption were measured on days 0,3,6-15,17 and 21. The uterus (live fetuses and early or late resorptions) and ovaries (corpora lutea) of the dams were studied and fetuses examined for weight, sex and external abnormalities. Alternate fetuses had the head and viscera examined, while the remained had the viscera and skeleton studied.

RESULTS

Maternal toxicity
Two animals at 150 mg/kg/day had seizures on single days. Hyperactivity and salivation also occurred sporadically. Doses above 60 mg/kg produced a transient decrease in body weight (3-8g over 2-3 days) corresponding to a reduction in food and water intake.

Embryo-fetal toxicity
1 control dam had complete resorption while 120 and 150 mg/kg/day groups had resorption in 3 and 2 dams respectively. No significant changes were noted for implantation sites, embryonic death or number of live fetuses (although postimplantation loss in HD was elevated at 22% vs 15% in control, Table 56). Mean fetal weight was somewhat reduced in 120 or 150 mg/kg/day groups (8-10%, about 0.4g) and there was a dose related decreased ossification of metatarsals for MD and HD groups (Table 56). The sponsor considers these the result of maternal toxicity. Malformations were seen in 3 litters (6 fetuses) @ 150 mg/kg/day and 1 litter (1 fetus) @ 120 mg/kg/day. In the HD group 2 fetuses in 1 litter had digit malformations consistent with the preliminary study, while the others showed cardiovascular and neural tube defects which may be incidental.

EVALUATION

Although a previous study (TW020BA part A) concluded that 30 mg/kg/day for E7-E8 and 150 mg/kg/day administered on days E9-E16 caused abortion (5/5 dams), a comparable effect was not observed in this study using 20/150 mg/kg/day. The incidence of digit malformations was also much lower than in the preliminary oral teratogenicity study which utilized 30/150 mg/kg/d (1/22 litters vs 3/10). The dose of 30 compared to 20 mg/kg/day may therefore have been influential in terms of digit malformations.
Fetal weight was reduced @ 120 and 150 mg/kg/d, but not

significantly, and post-implantation loss was increased @ 150 mg/kg/d.

**Investigative teratology study in the rat (report TW024BA)**
**Second investigative teratology study in the rat (report TW026BA)**

DOSE SELECTION

These studies investigated the timing and dose-relationship of ROP-induced teratogenicity using:
a) 150 mg/kg/day starting E8, 9, 10, 11 or 12 (and continuing to E15)
b) From E10-E15 animals were administered doses of 60-150 mg/kg/day

a) Timing study
10/group. Clinical signs in dams were consistent with those described above at a dose of 150 mg/kg/day.

RESULTS

| Group | Days dosed | Litters affected | Fetuses affected | Fetuses with digits affected | Other |
|---|---|---|---|---|---|
| 1 | - | | | | |
| 2 | 8-15 | 1 | 2 | 2 | 1 dam resorbed litter |
| 3 | 9-15 | 3 | 3 | 2 | |
| 4 | 10-15 | 2 | 2 | 2 | |
| 5 | 11-15 | | | | |
| 6 | 12-15 | 2 | 2 | 1 | |

Except for group 4, post-implantation loss was similar (around 12% vs 24% in gp 4), but the number of late embryonic deaths was higher in treated groups 2-4.

| Group | Embryo deaths | |
|---|---|---|
| | Early | Late |
| 1 | 12 | 0 |
| 2 | 3 | 8 |
| 3 | 6 | 7 |
| 4 | 7 | 18 |
| 5 | 10 | 4 |
| 6 | 10 | 3 |

b) Dose response study
20/group treated from E10-E15

| Group | Days 10-15 dose (mg/kg) | Litters affected | Fetuses affected | Fetuses with digits affected |
|---|---|---|---|---|
| 1 | - | | | |
| 2 | 60 | | | |
| 3 | 90 | 1 | 1 | |
| 4 | 120 | | | |
| 5 | 150 | 3 | 3 | 3 |

While the overall post-implantation loss rate for groups 1-4 was similar, the number of late deaths was dose-related and the HD group showed an overall increase in postimplantation loss:

| Group | Days 10-15 dose (mg/kg) | Postimplant loss % | Fetal wt g | Embryonic deaths | |
|---|---|---|---|---|---|
| | | | | Early | Late |
| 1 | - | 6 | 4.54 | 14 | 0 |
| 2 | 60 | 7 | 4.48 | 14 | 0 |
| 3 | 90 | 9.5 | 4.45 | 17 | 2 |
| 4 | 120 | 5.7 | 4.37 | 9 | 3 |
| 5 | 150 | 13.3 | 4.16 | 11 | 18 |

EVALUATION OF RAT TERATOLOGY STUDIES

The sponsor notes that literature reports indicate that the sensitive period for induction of digit malformations by physical (eg vascular clamping) or chemical (eg caffeine) means lies between E11 and E15. Thus, the differences between the preliminary (TW020BA) and definitive (TW023BA) teratology studies, which differed in the frequency of digit malformations, has been interpreted by the sponsor to be due to a difference in the day of initiation of 150 mg/kg/day dosing (days 8-15 only in the latter study, and days 9, 10 or 11 in the former). It is suggested that transient cardiovascular effects of ROP may result in a teratogenic effect that subsides with time. Subsequent investigative studies (TW024BA) were interpreted to indicate that 150 mg/kg/day was most teratogenic on day E10. A dose-response study showed that when administered from E10-E15, only 150 mg/kg/day was teratogenic.

However, the data are also consistent with the initial dose of ROP being important, i.e. a dose of 30 mg/kg in the preliminary study and 20 mg/kg in the definitive study. The investigative study was too small to distinguish the most sensitive period for teratogenicity since 1-3 litters (2 or 3 fetuses) were affected when dams were treated at times between E8 and E12.

These studies indicate that 150 mg/kg, and possibly 30 mg/kg can be teratogenic in rats when administered on specific days of gestation. 150 mg/kg approximately doubled the rate of postimplantation loss and decreased fetal weight (~10%).

**Dose-range study of SK&F 101468-A in pregnant rabbits (report TP003BA)**

DOSE SELECTION

A prior limiting toxicity study in pregnant rabbits employed gavage administration of 88, 131, 175 and 263 mg base/kg/day (= 100, 150, 200 and 300 mg salt/kg/day). 1 F/gp. The 300 mg/kg animal died in approx 6 hours, with rapid respiration and convulsions. All other doses produced hyperactivity and foot stomping, with decreased food consumption and body weight. Fetuses were alive, but the CNS effects were limiting to 150 mg/kg/day. The dose-ranging study thus used the following doses (mg base/kg/day by gavage):

Control (water); ROP 10, 25, 50, 75, 100 and 150 mg/kg/d

METHODS

Species:
New Zealand White rabbit, 6/group, pregnant.

Treatment:
One treatment daily for days 6-18 of gestation. The animals were sacrificed on day 29.

Observations:
Body weight, food consumption and clinical signs were recorded daily. Blood samples were taken on day 12 at 2, 4 and 6 hrs post-treatment from 25-75 mg/kg groups. Animals were sacrificed on day 29 and ovaries (for corpora lutea), uterus, fetuses (viscera and skeleton) were examined.

RESULTS
a)Maternal toxicity

Clin signs/mortality. Mortality was observed from day 6-13 in the following groups: 10 or 25 mg/kg (0%), 50 mg/kg (33%), 75 (50%), 100 (50%), 150 (100%). 1 animal in the 25 mg/kg and 75 mg/kg groups resorbed fetuses. All treated groups displayed hyperactivity and foot stomping. Rapid respiration often preceeded death, and tremors, vocalization, convulsions or prostration were often observed.

Body weight/food consumption. 10 mg/kg depressed food consumption and weight gain for day 7 while higher doses reduced intake for 2 or more days. These effects returned to normal and 10 or 25 mg/kg

treated animals approached control weight values, while higher doses remained below control levels.

b) Fetal toxicity

The frequency of postimplantation death was higher than historical controls (12%) in the 25, 75 and 100 mg/kg/day groups (22-23%). In this study, though, the control group had an abnormally low postimplant mortality. All fetuses were born live, without change in the proportion of M:F or external development. The medial lobe of the lung was absent in one fetus at 50 mg/kg/day and two animals at a two-fold higher dose. The incidence of this abnormality was noted as being high in historical controls.

c) Plasma drug concentrations

See Table 57. Determinations were made on day 12 of pregnancy. Although PK parameters could not be calculated there was increased exposure with dose for ROP, with SKF 104557 levels being greater than the parent. SKF 89124 levels were usually <MQL.

**Developmental toxicology study of SK&F 101468-A in pregnant rabbits** (report TP004BA)

DOSE SELECTION

In the rabbit dose-ranging study ROP was administered at 10, 25, 50, 75, 100 and 150 mg/kg/day p.o. (report TP003BA). Mortality of does was observed above 25 mg/kg/day, and post-implantation deaths were seen at 25 mg/kg/day. For the definitive study ROP was administered at a HD of 20 mg/kg/day for gestation days 6-18, with lower doses of 1 and 5 mg/kg/day for dose-response determination.

METHODS

Species:
New Zealand White rabbit
7-8 months of age, mean 3.7 kg, 20/group, mated.

Treatment:

One treatment daily by gavage for days 6-18 of gestation. The animals were sacrificed on day 29.

Observations:
Body weight, food consumption and clinical signs were recorded daily. Animals were sacrificed on day 29 and all fetuses were examined for abnormalities of viscera and skeleton.

RESULTS

a)Maternal toxicity

Clinical signs/mortality. Mortality was only seen in the HD group (2/20 died) after 2 or 3 doses, and followed rapid respiration and hyperactivity. All groups exhibited dose-related clinical signs such as hyperactivity, foot stomping and rapid respiration.

Body weight/food consumption
HD females had a significant drop in body weight (-2.6% to 0.1%) over days 7 to 11 associated with up to a 50% (day 7)reduction in food consumption. Both effects decreased with time, but the HD rabbits stayed below mean control weight gain up to the end of the study. MD animals showed a slight, but not significant, transient effect on food consumption after initial dosing.

b) Embryo-Fetal toxicity

No dose-related toxicities were observed. No significant changes occurred in corpora lutea number, implants, live fetuses number and weight, or sex distribution. 1 MD fetus had an umbilical hernia. The number of animals with absence of the lung medial lobe was elevated (3.3-5.9% vs 1.3% in controls) in treatment groups but was within historical range for controls (0.5-9%). Cardiovascular abnormalities were limited to 1 HD fetus (enlarged aorta/septal defect) and 1 control (enlarged aorta, septal defect and hypoplastic pulmonary artery. No consistent skeletal abnormalities were observed in treated groups compared to controls although more fetuses in LD and MD treatment groups possessed a cervical rib and 1 HD fetus was missing a cervical vertebrae.

EVALUATION

At the clinical signs were observed, with significant reduction in food consumption and body weight gain after initiation of dosing, as well as some mortality. The MD and LD caused clinical

signs without other toxicity. No drug-related teratogenicity was apparent.

**Perinatal/postnatal study of SK&F 101468-A in female rats (report TP-0016/SKF-101468/1)**

DOSE SELECTION

10 mg/kg/d decreased maternal body weight gain and food consumption, and reduced pup weight gain during laction in previous studies. 0.1 and 1.0 mg/kg/d were not expected to impair lactation or pup weight gain.

METHODS

Species:
Charles River CD rat 70-80 days of age, 200-400g, 22/group

Treatment:
ROP 0, 0.1, 1, 10 mg/kg/d by gavage in water for days E15-P21
Culling to 8 pups by random selection occurred on day 7.

Observations:
Females were monitored for body weight, food consumption and physical signs. F1 offspring had body weight and physical condition examined, neurobehavioral development assessed and reproductive performance determined.

RESULTS

Maternal effects were primarily ptosis. No decrease in food consumption or body weight was observed, in fact weight gain increased during days 7-21 of lactation at 10 mg/kg. No adverse effects on delivery, # pups or % post implant loss were noted.

An increase in pup weight (5-8%) was observed in the 10 mg/kg/day group at P1-2, then this group had reduced weight gain for days 4-14 resulting in significantly decreased weight (15-20%) at day 21. At 7-8 weeks weights were comparable to controls. LD and MD were similar to controls at all time points.

A small decrease (about 0.5 days) was observed in time to pinna unfolding, incisor eruption and eyelid opening, that corresponded to an increase in weight in early postnatal days at 10 mg/kg/d. Balano-preputial skinfold separation and vaginal opening were slightly delayed(1.7 and 0.6 days). No significant differences were noted for negative geotaxis reflex or pupillary reflex.

Abnormalities in neurological development observed were a reduction in startle reflex (up to 25%) for female rats at 1 and 10 mg/kg/d groups at day 29 and a reduction in the latter group response at day 62-64. Males were not different from control. No significant changes were noted for passive avoidance, swim maze performance or motor activity for F1 animals. Reproduction was not altered in F1 animals (estrus cycle, fertility, # corpora lutea and implantation sites.

EVALUATION

In a dose-ranging study 20 mg/kg/day produced a profound reduction in pup weight at day 21 (up to 47%) and depression of startle reflex by 20-50% (report TP009BA), suggesting a drug-related effect. Startle rflex effects did not correlate with body weight, raising the possibility that CNS DA-receptor stimulation or subsequent endocrine changes are reflected in this neurological abnormality. The large reduction in pup weight gain at 20 mg/kg/d may be ascribed to decreased lactation. The observation of alteration in startle reflex at 10 mg/kg/d was suggested by the sponsor to possibly reflect a direct action of ROP during development (rather than postnatal since administration of DA agonists usually increases this reflex) or reduced prolactin.

## SPECIAL TOXICITY STUDIES

**SK&F 101468-A and L-DOPA: Oral Study for Toxicological and Embryo-Fetal Developmental Effects in Rabbits. (Doc # TP-1010/SKF 101468/1)**

DOSE SELECTION

SKF 101468
In a rabbit Segment II dose-ranging study ROP was administered at 10, 25, 50, 75, 100 and 150 mg/kg/day p.o. (report TP003BA) and at 1, 5 and 20 mg/kg/day in a definitive study (report TP004BA) for gestation days 6-18. 10 mg/kg/day produced hyperactivity (pharmacological action) and transient (1-2 day) slight decreases in food consumption and body weight gain. 20 mg/kg/day produced hyperactivity, with mortality in 2 animals after 2-3 days. Food consumption was reduced for the initial 3 days with concomitant reduction of body weight gain (-2% over first 2 days, resulting in total gain being below control throughout study). The Cmax following 25 mg/kg ROP was about 385 ng/ml (Table 57), approximately 10-fold higher than the human plasma level at the max recommended dose (8 mg/kg t.i.d.).

L-DOPA
A literature study referenced employed L-DOPA at doses of 75, 125 or 250 mg/kg/day, with some fetal toxicity but no data on maternal toxicity. A sponsor study of 10 mg/kg/day ROP with 40, 125 and 250 mg/kg/day L-DOPA (SK&F 101468 and L-DOPA Maximum Tolerated Oral Dose Study in rabbits; report # TP-1009/SKF 101468/1) in 2 non-mated animals per group was performed. This study lacked a control group and pre-treatment data, but appeared to indicate that in non-pregnant animals only the HD L-DOPA group showed impairment of food consumption and weight gain. Clinical signs (hyperactivity, foot stomping) appeared comparable to ROP alone in all groups.

METHODS

Species:
New Zealand White rabbit, 23/group, mated.

Doses (mg/kg/day by gavage):
Control (methylcellulose); ROP 10; L-DOPA 250 ; ROP 10 + L-DOPA 250

Treatment:
One treatment daily for days 6-20 of gestation. The animals were sacrificed on day 29.

Observations:
Functional Observational Battery (responsiveness, behavior and general clinical condition), body weight, food consumption. Day 29; ovaries (for corpora lutea), uterus, fetuses (internal organs, skeleton and brain examined).

RESULTS
a) Maternal Toxicity

Clin signs/mortality. Three does in the combination group died. Exaggerated clinical signs were observed in the combination group (in addition to hyperactivity and evasive behavior due to ROP and foot stomping produced by L-DOPA) including salivation, lacrimation, increased pupil size and more rapid or shallow respiration. Respiratory responses appeared to be associated with mortality.

Body weight/food consumption. The use of 3-day averaging obscured a transient slight reduction in body weight following treatment with ROP. The magnitude appeared similar to that occurring in the

segment II dose ranging study described above (TP)003BA. Food consumption was significantly reduced only in the combination group but ROP alone had a small transient effect as noted in dose-ranging studies.

No effects were noted on corpora lutea, implantations, resorptions or fetal number.

b) Fetal Toxicity

Body weight. All L-DOPA treated groups had significantly (-10%) reduced body weights (female fetuses treated with L-DOPA alone did not reach significance but were below historical controls). The magnitude was similar in groups +/- ROP.

Malformations. L-DOPA greatly increased the number of malformations observed, primarily of digits, with some sterebrae abnormalities (Tables 59 and 60). Additional administration of ROP did not elevate the # litters affected but the number of fetal malformations were increased. Brachydactyly and absence of claws were increased in the combined group vs L-DOPA alone, but not significantly. Adactyly was only observed in the combined dose group (3/15 litters, 10/137 fetuses; Table 60) and was therefore highly significantly increased. The significant increase in agenesis of lung medial lobe observed in the combination dose group may be incidental as the sponsor notes a high historical control rate.

EVALUATION

The use of single doses of L-DOPA and ROP makes the study of limited value as far as negative observations are concerned. However, the occurrence of adactyly in 20% litters and 7% of fetuses only in the combined group indicates a positive teratogenic interaction, in contrast to the sponsor's conclusion of no additive effect. The sponsor notes studies suggesting hypotension (e.g. from Ca channel blockers or uterine vessel clamping) leading to decreased uterine blood flow produces vasodilation, edema, hemorrhage and malformations in autopods. On this basis, brachydactyly and adactyly may result from the same mechanism, with adactyly resulting from the additive pharmacological action of ROP and L-DOPA as evidenced by additive clinical signs.

**Influence of SK&F 101468-A on Leydig cell LH receptor profile in rats (report TP1006/SKF-101468/1)**



DOSE SELECTION

SKF 101468A was administered at 50 mg/kg, the HD in carcinogenicity studies where Leydig cell tumors were observed.

METHODS

Species: M sprague-Dawley rats, about 15 weeks of age

Doses (mg/kg/day by gavage): 0, 50 10 M/group

Treatment: Daily gavage for 8 days

Observations: Day 8 LH, PRL, testosterone, LH receptor density in Leydig cell enriched cell fraction (membrane prep).



RESULTS

PRL decreased >90%; control 26.5 ng/ml, ROP treated 0.2
LH unaffected; control 2.8 ng/ml, ROP treated 2.7
T decreased; control 1.6 ng/ml, ROP treated 0.8
LH receptors decreased; control 0.8 fmol/mg, ROP treated 0.27

EVALUATION

The mechanism of Leydig cell proliferation following hypoprolactinemia is unclear although the proliferative response appears consistent. Several studies have indicated elevated LH levels, which may increase in response to LH receptor down regulation and decreased T production, are likely to be the mitogenic stimulus. In the current study LH levels did not increase, but a published study indicated that after 1 week only PRL and T are reduced by a DA agonist (mesulergine) and LH levels do not rise until 4 weeks of treatment [Prentice et al., Medical Toxicology 1991, Bolt, deWolf and Henderson (eds), 197-204, Springer-Verlag, NY].

TABLE 53 F Fertility study

P = day pregnancy

| Dosage Groups | | | | | |
|---|---|---|---|---|---|
| Group | Treatment with SK&F 101468 (mg/kg/day) | | | | Experimental Numbers |
| | S13-P0-1* | P0-P8 | P9-P20 | P21-L20 | |
| 1 | 0 | 0 | 0 | 0 | 1001-1030 |
| 2 | 5 | 5 | 5 | 5 | 2001-2030 |
| 3 | 50 | 5 | 50 | 10 | 3001-3030 |
| 4 | 100 | 5 | 100 | 20 | 4001-4030 |

* P0-1: the day before conception



TABLE 54

| Dose mg/kg | No. Litters | Corpora Lutea | Stained Implantation Sites | Live Fetuses | Pre-Implantation Loss (%) | Post-Implantation Loss (%) | Mean Fetal Weight (g) | Sex Ratio (%M) |
|---|---|---|---|---|---|---|---|---|
| Control | 12 | 12.5 | 10.6 | 8.8 | 15.4 | 20.6 | 4.27 | 43.9 |
| SD | | 1.4 | 3.3 | 4.0 | 24.0 | 22.3 | 0.46 | 21.0 |
| 5 | 13 | 13.5 | 12.9 | 11.5 | 4.8* | 11.5 | 4.43 | 40.6 |
| SD | | 1.5 | 1.4 | 1.9 | 4.6 | 8.9 | 0.37 | 19.8 |
| 50/5/50/10 | 13 | 14.0 | 11.1 | 9.2 | 17.9 | 16.7 | 4.38 | 48.6 |
| SD | | 3.7 | 2.2 | 3.0 | 19.5 | 19.4 | 0.41 | 12.0 |
| 100/5/100/20 | 12 | 14.5 | 13.3 | 12.1 | 8.3 | 9.1 | 4.14 | 47.6 |
| SD | | 2.2 | 2.5 | 2 | 11.1 | 6.5 | 0.41 | 18.5 |

* N = 12

[redacted]: Incidence of Embryonic Deaths

| Group | No. of Embryonic Deaths | | % Embryonic Deaths that were 'late' |
|---|---|---|---|
| | Early | Late | |
| 1 | 6 | 0 | 0 |
| 2 | 5(+17*) | 1 | 17(5**) |
| 3 | 8 | 8 | 50 |
| 4 | 12 | 21 | 64 |

*aborted fetuses **if aborted fetuses included

Embryotoxicity in Group 4 was further indicated by the apparently lower mean fetal weight (4.08 ± 0.53 g vs 4.63 ± 0.46 g in controls).



[redacted]: Incidence of malformations

| Group | Incidence of Malformations | |
|---|---|---|
| | No. of fetuses (Affected/total) | No. of litters (Affected/total) |
| 1 | 0/111 | 0/10 |
| 2 | 5/93 | 1/8 |
| 3 | 14/96 | 2/9 |
| 4 | 12/87 | 3/10 |

Preliminary Rat teratology study

Table 55

Table 5

Pregnancy Data

101468/033/RAT

Group Mean Values ± SD

| Dose mg/kg | Corpora Lutea | Stained Implantation Sites | Embryonic Deaths | | Live Fetuses | Pre-Implantation Loss(%) | Post-Implantation Loss(%) | Mean Fetal Weight(g) |
|---|---|---|---|---|---|---|---|---|
| | | | Early | Late | | | | |
| Control (n=23) | 13.4 ± 1.31 | 12.0 ± 2.85 | 1.4 ± 1.41 | 0.1 ± 0.29 | 10.7 ± 3.30 | 10.2 ± 19.51 (n=22) | 15.5 ± 22.98 | 4.39 ± 0.29 (n=22) |
| 20 (n=24) | 13.8 ± 1.69 | 12.1 ± 2.72 | 0.5 ± 0.66 | 0.1 ± 0.28 | 11.6 ± 2.80 | 11.6 ± 21.10 (n=23) | 4.80 ± 7.45 | 4.44 ± 0.27 |
| 20/60 (n=25) | 13.6 ± 2.57 | 12.2 ± 1.78 | 0.9 ± 0.91 | 0.1 ± 0.33 | 11.2 ± 1.93 | 9.20 ± 11.05 | 8.55 ± 8.45 | 4.38 ± 0.24 |
| 20/90 (n=24) | 13.3 ± 2.43 | 11.6 ± 1.77 | 0.8 ± 1.05 | 0.1 ± 0.28 | 10.7 ± 1.90 | 11.7 ± 14.55 | 7.70 ± 9.00 | 4.32 ± 0.27 |
| 20/120 (n=23) | 13.7 ± 1.56 | 12.2 ± 1.81 | 2.2 ± 4.00 | 0.3 ± 0.56 | 9.7 ± 4.26 | 10.1 ± 11.86 | 20.7 ± 32.33 | 4.15 ± 0.25 (n=20) |
| 20/150 (n=24) | 13.9 ± 2.48 | 11.9 ± 2.89 | 1.5 ± 2.19 | 0.7 ± 0.96 | 9.7 ± 3.50 | 13.4 ± 20.82 | 22.1 ± 27.31 | 4.01 ± 0.28 (n=22) |

Pivotal rat teratology study

Table 56

TABLE 56

101468/033/RAT

Summary of Skeletal Anomalies
Number of Litters Affected

| Dose mg/kg | No. of Litters Examined | Reduced/Absent Ossification | | | | Vertebrae | | | Ribs | | Astra-galus Present |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cervical Vertebrae | 5th Ster'brae | Forelimb Metacarps | Hindlimb Metatars | Lumbar Reduc[a] | Lumbar Addtl[b] | Bipart Centra | 14th Ribs | 14th Dots | |
| Control | 22 | 15 | 6 | 1 | 7 | - | - | 6 | 1 | 10 | 8 |
| 20/10 | 24 | 13 | 6 | - | 7 | 1 | - | 8 | 1 | 11 | 15 |
| 20/60 | 24 | 13 | 6 | - | 4 | - | 1 | 5 | - | 10 | 11 |
| 20/90 | 24 | 13 | 4 | - | 8 | 1 | - | 8 | 1 | 10 | 7 |
| 20/120 | 20 | 10 | 4 | - | 11 | 2 | - | 6 | 1 | 7 | 3 |
| 20/150 | 22 | 15 | 7 | 4 | 16 | - | 1 | 9 | - | 6 | 1 |

[a] Number of lumbar vertebrae reduced from 6 to 5
[b] Number of lumbar vertebrae increased from 6 to 7

days E6, E7

days 8 - E15

Rivolat Rat Teratology Study TWO73BA

Table 56A

000230

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Pod 7

Table 9

SK&F 101468-A

Dose-Range Study in Pregnant Rabbits

Plasma Concentration (μmol $l^{-1}$)

Summary

n = 1 pregnant rabbit / gp

| Dose (mg/kg/day) | Female No. | Hrs. Post-Treatment on day 12 of Pregnancy 2 | 4 | 6 |
|---|---|---|---|---|
| SK&F 101468 | | (ng/ml) | | |
| 25 | 18 | (385) 1.48 | 0.83 | 0.50 |
| 50 | 23 | (1628) 6.26 | 5.20 | 3.24 |
| 75 | 30 | (1180) 4.54 | 9.62 | 11.20 |
| SK&F 89124 | | | | |
| 25 | 18 | ND | NQ | ND |
| 50 | 23 | ND | 0.11 | NQ |
| 75 | 30 | NQ | 0.13 | NQ |
| SK&F 104557 | | | | |
| 25 | 18 | (1062) 4.60 | 3.03 | 0.91 |
| 50 | 23 | (4782) 20.7 | 24.6 | 11.9 |
| 75 | 30 | (3396) 14.7 | 30.8 | 32.8 |

ND = Not Detected
NQ = Not Quantifiable

Contains data only from rabbits that had normal litters at term. Data from other animals (non-pregnant or totally resorbed litters) are in Appendix, Section E.

Range finding in rabbits Day 12
Seq II

Table 57,

70



Table 10

SK&F 101468-A

Developmental Toxicology Study in Rabbits

Examination of Fetal Skeletons

Summary

| Group | Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|
| No. Litters Examined | 20 | 18 | 19 | 16 |
| No. Fetuses Examined | 147 | 137 | 136 | 105 |
| % Litters with* | | | | |
| Skull Variations | | | | |
| Open Anterior Fontanel (sl.) | 0 | 0 | 5.3 | 0 |
| Extra Sutures | 10.0 | 11.1 | 26.3 | 0 |
| Incomplete Ossification | 5.0 | 5.6 | 0 | 0 |
| Vertebrae Variations | | | | |
| Cervical | | | | |
| Cervical Rib | 5.0 | 38.9 | 10.5 | 6.3 |
| <7 Vertebrae | 0 | 0 | 0 | 6.3** |
| Thoracic | | | | |
| Hypoplastic Arch | 0 | 0 | 0 | 6.3 |
| Hypoplastic Center | 5.0 | 0 | 0 | 6.3 |
| Absent Arch | 5.0 | 0 | 0 | 0 |
| Sternal Center Variations | | | | |
| <6 | 45.0 | 44.0 | 47.4 | 43.8 |
| Hypoplastic | 80.0 | 77.8 | 84.2 | 81.3 |
| Fused | 5.0 | 11.1 | 0 | 0 |
| Rib Variations | | | | |
| Floating Rib | 5.0 | 0 | 0 | 0 |
| 13 Ribs | 100.0 | 88.9 | 100.0 | 100.0 |
| Metacarpals - <5 | 15.0 | 15.6 | 15.8 | 12.5 |

Data were not statistically analyzed.

* Percentage litters containing any fetus(es) with the indicated variation.

** Also had cervical center missing and cervical arch incompletely ossified.

Rabbit Leg II

Table 17

Table 8

TOXICOLOGY STUDY NO. G95058

Total Incidence of Fetal (F1) Malformations - Summary (s)
(mean +/- SEM)
(n = number of litters)
(includes all live and dead fetuses at term)

| Male (F0) Dose Group<br>Test Used: | Mean % of fetuses per litter with malformations<br>KW+ | Malformed fetuses/ total fetuses<br>NT | Litters with at least 1 malformed fetus/ total litters<br>FE+ |
|---|---|---|---|
| 0 mg/kg/day | 2.8 +/- 1.33 (n = 22 ) | 5 /189 | 4 /22 |
| 10 (a) mg/kg/day | 3.0 +/- 1.27 (n = 21 ) | 5 /194 | 5 /21 |
| 250 (b) mg/kg/day | 16.0 +/- 4.74* (n = 22 ) | 35 /181 | 13 /22* |
| 10+250 (c) mg/kg/day | 24.5 +/- 8.30*,^ (n = 15 ) | 34 /137 | 8 /15 |

(s) = Malformations are those fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance, and these are listed individually in Table 9.
(a) = SK&F 101468-A
(b) = Levodopa
(c) = SK&f 101468-A and Levodopa
* = Pairwise analysis indicated a significant difference from the Control
^ = Pairwise analysis indicated a significant difference between the 10 mg/kg/day and 10 + 250 mg/kg/day groups.







Table 59

RoP + L-DoPA

TOXICOLOGY STUDY NO. C95058

Incidence of Individual Fetal (F1) Observations - Summary (a)

Mean % per Litter (+/- SEM)

(Fetuses Affected / Fetuses Examined) (Litters Affected / Litters Examined)

| Observation | 0 Control mg/kg/day | 10 (a) mg/kg/day | 250 (b) mg/kg/day | 10 +250 (c) mg/kg/day |
|---|---|---|---|---|
| §External Extremities - Amury (FE-) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.57 +/- 0.57 (1 /181 ) [1 /22 ] | 0.00 +/- 0.00 (0 /137 ) [0 /15 ] |
| §External Extremities - Adactyly (FE+) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.00 +/- 0.00 (0 /181 ) [0 /22 ] | 7.19 +/- 5.36*,^,+ (10 /137 ) [3 /15 ] † |
| §External Extremities - Brachydactyly (FE+) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 4.67 +/- 2.61 (11 /181 ) [4 /22 ] | 7.21 +/- 3.20*,^ (11 /137 ) [5 /15 ] |
| §External Extremities - Oligodactyly (FE-) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.41 +/- 0.41 (1 /181 ) [1 /22 ] | 2.44 +/- 1.67 (3 /137 ) [2 /15 ] |
| §External Extremities - One or More Claw(s) Not Evident (FE+) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 6.41 +/- 3.42* (15 /181 ) [5 /22 ] | 10.23 +/- 4.71*,^ (15 /137 ) [5 /15 ] |

(a) = Malformations are those fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance. These are identified by a number (§). All other fetal observations represent retardations in development, transitory alterations or permanent alterations not believed to adversely affect survival, growth, development, function, longevity, or external appearance.
(a) = SKAF 101468-A, (b) = Levodopa , (c) = SKAF 101468-A and Levodopa

Pairwise comparison results for the proportion of litters affected indicated by *,^,+ (see page B1)

* Drug vs group 1 (control)
^ Group 2 vs Gp 4
+ Group 3 vs Gp 4

Table 6 (continued)

RO1 + L DOPA

Table 9 (Cont'd)

TOXICOLOGY STUDY NO. 098058

Incidence of Individual Fetal (F1) Observations - Summary (s)

Mean % per Litter (+/- SEM)

(Fetuses Affected / Fetuses Examined) (Litters Affected / Litters Examined)

| Observation | 0 Control mg/kg/day | 10 (a) mg/kg/day | 250 (b) mg/kg/day | 10 +250 (c) mg/kg/day |
|---|---|---|---|---|
| Great Vessel - Enlarged Ascending Aorta (FE-) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.41 +/- 0.41 (1 /181 ) [1 /22 ] | 0.00 +/- 0.00 (0 /137 ) [0 /15 ] |
| Great Vessel - Enlarged Aortic Arch (FE-) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.41 +/- 0.41 (1 /181 ) [1 /22 ] | 0.00 +/- 0.00 (0 /137 ) [0 /15 ] |
| Heart - Enlarged Ventricle(s) (FE-) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.51 +/- 0.51 (1 /181 ) [1 /22 ] | 1.11 +/- 1.11 (1 /137 ) [1 /15 ] |
| Heart - Enlarged Atrium(s) (FE-) | 0.00 +/- 0.00 (0 /189 ) [0 /22 ] | 0.00 +/- 0.00 (0 /194 ) [0 /21 ] | 0.51 +/- 0.51 (1 /181 ) [1 /22 ] | 1.11 +/- 1.11 (1 /137 ) [1 /15 ] |
| Lung - Medial Lobe Not Evident (FE+) | 0.41 +/- 0.41 (1 /189 ) [1 /22 ] | 0.95 +/- 0.95 (2 /194 ) [1 /21 ] | 1.30 +/- 1.30 (2 /181 ) [1 /22 ] | 4.41 +/- 2.05*,^,+ (6 /137 ) [3 /15 ] |

(s) = Malformations are those fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance. These are identified by a number (#). All other fetal observations represent retardations in development, transitory alterations or permanent alterations not believed to adversely affect survival, growth, development, function, longevity, or external appearance.

(a) = SK&F 101468-A, (b) = Levodopa , (c) = SK&F 101468-A and Levodopa

Pairwise comparison results for the proportion of litters affected incidated by *,^,+ (see page 81)

Table 6 — cont

A1 + L-DOPA

## SUMMARY AND EVALUATION

### Pharmacodynamics

*Mechanism of Action*

Ropinirole is being developed for the treatment of Parkinson's disease, both as monotherapy and combination therapy with L-DOPA. As a direct DA receptor agonist, ROP is intended to provide symptomatic relief by compensating for loss of dopamine receptor activation which results from degeneration of nigrostriatal neurons and reduced DA release. ROP has selectivity for D2-type receptors, which are highly expressed in striatum, as compared to D1-type receptors.

PRIMARY PHARMACOLOGICAL ACTIONS

Note: Except where specific reference is made to D3 and D4 receptors, the term D2 receptor indicates the D2-family which includes D2, D3 and D4 receptors with splice variants.

*In vitro activities*

In rat cortical membrane binding studies ROP displayed selectivity for D2-type (D2/D3/D4) receptors as compared to D1-type receptors (>10-fold), but this selectivity was not examined in either cloned or native human receptors. A lack of ROP-related rat caudate adenylate cyclase activation at 10 $\mu$M was consistent with low affinity for rat D1 receptors. Data on inhibition of adenylate cyclase, the D2-receptor second messenger coupling, was not submitted. However, in a sponsor publication (Gallagher et al., J. Med. Chem. 28, 1533-1536, 1985) ROP was reported to not inhibit dopamine-stimulated adenylate cyclase activity at concentrations up to 100 $\mu$M. This result is indicative of the complex pharmacology of DA receptor subtypes and the difficulty of defining receptor profile between different tissues.

The potency of ROP for D2 receptors was moderate in both human and rat cloned receptors (about 1 $\mu$M) and much lower than for either bromocriptine or pergolide (>40-fold). In fact ROP, bromocriptine and pergolide possessed equivalent or higher affinity for D3 receptors as compared to D2. D4 activity approximated that at D2 receptors in the case of ROP (the other agonists were not examined). These and other binding studies were performed using receptors expressed in CHO or HEK 293 cells. While cloned receptors can contribute to subtype analysis, there is the potential for significant differences in pharmacology compared to native receptors. In cells lines, for instance, there may be poor G-protein binding that leads to expression of

receptors in a low-affinity conformation. The divergence between cloned and native D2 high affinity site potency for ROP (29 nM in rat striatum and human caudate compared to values of 948 and 1380 nM in cloned rat and human D2 receptors respectively) illustrates the difficulty of making comparisons between such studies, although the order of potencies for DA agonists were similar in both types of experiments.

With regard to other receptors, ROP had IC50 values of approximately 10 $\mu$M for $\alpha$2 adrenoceptors and lower affinity for 5HT2 receptors. Opioid activity was somewhat greater, with $\kappa$ (447 nM Ki) and $\mu$ (700 nM Ki) activity in guinea pig brain, but naloxone did not affect the behavioral activity of ROP suggesting little functional significance. While the affinity of ROP for these receptors was low, the relative selectivity vs D2 is difficult to determine due to the variability in DA receptor Ki values between tissues studied. ROP was more selective for DA receptors vs non-DA receptors than bromocriptine or pergolide since both of the latter compounds had high affinity for $\alpha$-adrenoceptors (Ki <60 nM) and 5-HT1 receptors (bromocriptine Ki<100 nM and pergolide <200 nM respectively).

Overall, ROP can be considered to be a D2-type (compared to D1) agonist with moderate potency and selectivity, and a relative subtype selectivity of D3>D2=D4. The functional role of D3 and D4 receptors is currently unclear, although recent reports suggest that hypothermic responses of DA agonists may be related to D3 receptor stimulation. Because all the anti-Parkinsonian DA agonists have high affinity (and undefined efficacy) for D3 receptors, the relative contribution of these receptors to efficacy is not known. However, D3 expression in the striatum is much lower than for D2 suggesting the latter is the primary therapeutic target.

A functional test consistent with D2 receptor activation, ACh release from rat striatum, indicated that ROP is a potent (IC50 of 90 nM) nearly full agonist (85% of max response), while bromocriptine had lower efficacy (33% of max response). The correspondence between functional and native receptor potency suggests human caudate would be the tissue of choice to examine receptor affinity for ROP and metabolites. It is unclear why inhibition of adenylate cyclase activity was not observed since this should correlate with functional activity in the striatum.

*In vivo activities*

i) Mechanistic studies

In agreement with in vitro data, drug discrimination studies indicated that ROP, like other D2 agonists, had no effect in animals sensitized to a D1 agonist.

ROP and bromocriptine were examined for their abilities to alter monoamine turnover in mouse brain. No effects were noted for norepinephrine or serotonin, while dopamine metabolites, but not DA levels, were reduced. Decreased turnover is consistent with activation of D2 receptors on presynaptic DA neurons. Decreased firing of DA cells in the pars compacta of the substantia nigra observed following ROP or apomorphine administration also supports a presynaptic inhibitory action to decrease DA release. It should be noted that while binding data indicate ROP would be a postsynaptic DA receptor agonist in vivo, the presynaptic inhibitory action of ROP would lead to reduced DA levels at the postsynaptic DA receptor sites. This could be regarded as therapeutically useful if lowered DA conversion from L-DOPA, and thus reduced free radical generation, were involved in neurodegeneration. However, recent studies have cast doubt on the role of free radicals in Parkinson's disease.

In rats and mice with unilateral lesions induced by 6-OHDA, ROP caused a prolonged contralateral asymmetry or frank circling comparable to apomorphine, indicating direct agonism at the supersensitive DA receptors on the lesioned side. Amphetamine, due to its indirect effect, caused ipsilateral rotation. Unilateral injection into the striatum of normal rats also caused contralateral circling consistent with DA receptor activation. In this model, though, apomorphine was not active. This limited study could reflect greater agonist activity of ROP compared to apomorphine, but could also result from differing receptor pharmacology profiles.

Locomotor studies demonstrated biphasic actions of ROP, with hypoactivity at low doses and hyperactivity at high doses. Apomorphine generally had similar effects to decrease locomotion at low doses, but was not stimulatory. In contrast, climbing activity was stimulated by other DA agonist to a much greater degree than by ROP. Hypoactivity and hyperactivity data are postulated to reflect actions at presynaptic and postsynaptic receptors respectively, but the varying, interdependent, behavioral responses make a definitive link impossible to assess.

ii) Animal models of disease

Marmosets were treated with MPTP infused unilaterally into the s. nigra over 14 days. This administration produced a Parkinson's-like syndrome after several days with hypoactivity, bradykinesia, limb rigidity, and immobility of the face and neck which could be assessed by behavioral responses to stimulation. This model has the advantage of causing unilateral deficits, so the animals have residual functions, but drug studies have to take place over the last 7 days of infusion as recovery takes place subsequent to terminating MPTP administration. It is therefore possible that an interaction occurs with MPTP rather than solely at DA receptors

ROP, at an oral dose of 0.1 mg/kg, reversed (to about 60% of control) both locomotor and behavioral deficits induced by MPTP. Higher doses caused emesis in at least some animals, although 0.5 mg/kg was tolerated in one study. Using the latter dose it was observed that ptosis and cardiovascular effects of ROP declined with repeat dosing over 4 days but that reversal of Parkinson's-like symptoms was sustained. Unfortunately, this study employed essentially a maximal dose with the result that any tolerance to behavioral effects would have been difficult to detect, and stability of MPTP-induced deficits was not demonstrated for the appropriate treatment period. Another study employing s.c. dosing also suffered from only employing doses high enough to cause emesis. These studies with repeated doses were therefore consistent with a lack of tolerance observed in lesioned rats, but were of limited value. Very limited studies of L-DOPA and benserazide demonstrated a reversal of deficits, but no conclusion could be drawn with regard to efficacy relative to ROP.

ROP appeared to have a favorable profile in a model of dyskinesia, in which peri-oral dyskinesia was induced with a DA agonist. Both ROP and tiapride inhibited dyskinesia, although ROP was less efficacious. While inhibition of dyskinesia in this model may simply reflect receptor activity of the standard agonist, these data were supportive of only weak induction of stereotyped behavior, which may relate to human side effects, at doses that reduced MPTP-induced deficits in marmosets.

SECONDARY PHARMACOLOGICAL ACTIONS

D2 receptors are present on peripheral sympathetic nerve terminals. ROP was examined in two isolated preparations that assessed sympathetic activity: constriction of the rabbit isolated ear artery following electrical stimulation, and radiolabelled norepinephrine release from dog vascular tissues (saphenous vein and coronary artery). ROP inhibited sympathetic activity in both tissues. In the rabbit ear artery ROP had high affinity (EC50 < 100 nM) and was antagonized by a sulpiride, a known D2 antagonist. These data were similar to those observed using striatal tissue, suggesting that ROP would have similar effects on central and peripheral D2 receptors.

Tachycardia following stimulation of the cardiac accelerans nerve was utilized to examine sympatholytic effects of ROP in the anesthetized dog, monkey and cat. These studies consistently showed a depression of sympathetic drive, especially at low stimulation frequency. Antagonism by sulpiride in the dog was consistent with a D2 mechanism, corresponding to a reduction of transmitter release via presynaptic D2 receptors. In vitro studies of guinea-pig atria or whole heart also demonstrated a depressant action of ROP, with similar or lower potency compared

to propranolol, but the mechanism of depression was not defined. Although ROP can cause hypotension by reducing sympathetic reflexes, efficacy was limited even at low rates of activity and reduced at high stimulus frequency, suggesting a ceiling was likely to exist to the degree of hypotension produced in vivo.

Behavioral testing in the mouse and marmoset suggested that ROP may possess some anxiolytic activity, unlike bromocriptine, but that there was no rebound anxiogenic effect on withdrawal of chronic treatment. Correspondingly, no withdrawal effects were observed after chronic ROP administration in the cynomolgus monkey, nor did ROP affect withdrawal following morphine or barbiturate dependence.

ACTIVITY OF METABOLITES

SKF 89124, the major metabolite of ROP in rat but only a minor metabolite in human or monkey, had about 15 to 20-fold greater potency at D2 receptors in receptor binding and functional studies. Following IP dosing in rats lesioned with 6-OHDA, SKF 89124 was approximately equipotent with ROP. The glucuronide metabolite of SKF 89124 was not examined for receptor affinity, but is unlikely to have significant activity. In contrast to SKF 89124, SKF 104557, the major metabolite in humans, monkey and mouse, was about half as potent as ROP in binding assays with little activity functional assays in vitro or in vivo (1/30 to 1/100 of the activity of ROP). Since SKF 89124 and its derivatives only comprised about 10% of human ROP and metabolites in urine, and SKF 104557 was functionally of low activity, the metabolites of ROP are unlikely to contribute substantially to the pharmacological profile of ROP in humans. In the rat SKF 89124 could contribute to DA receptor activation.

**Safety Pharmacology**

In concordance with the sympatholytic activity observed in vitro and in vivo, ROP decreased blood pressure (BP), between -30 and -70 mmHg, in anesthetized spontaneously hypertensive rat(SHR), dog and cat. Heart rate responses were variable, probably due to the direct and indirect influences on sympathetic activity, but usually decreased with increasing dose. These effects were accompanied by decreased cardiac metabolic rate and coronary blood flow, presumably due to the fall in BP. A D2 receptor action was confirmed with antagonist studies. The human and monkey primary metabolite SKF 104557 was about 30-fold less active than ROP, and SKF 89124 was more potent, in agreement with isolated tissue studies. In contrast to the CNS effects of ROP, tolerance to the hypotensive actions occurred with 7-14 days oral treatment (followed by IV challenge). This appeared to be due to receptor/coupling down regulation since increased doses of ROP or

bromocriptine were no more effective. Additionally, a study using 40 mg/kg p.o. ROP b.i.d. for 2 days followed by 0.5 mg/kg IV indicated that plasma levels following the IV dose were higher than in rats that received only saline for 2 days prior to the IV dose, ruling out a change in PK parameters as a mechanism for cardiovascular tolerance to ROP.

Tilt-induced hypotension was utilized to model orthostatic hypotension, in anesthetized normal and SH rats. ROP significantly potentiated the duration of reduced BP by inhibiting the normal tachycardia reflex response and subsequent recovery of BP. At the highest dose tested, 50 μg/kg IV, the normal tachycardia response was absent and a bradycardia was observed. Tilt-induced hypotension with bradycardia is believed to be associated with syncope in humans (Nwosu et al., Am Heart J., 1994, 128: 106-113). The response can be induced by a variety of sympatholytic agents eg isoproterenol. It may be mediated by a mechanoreceptor-mediated vagal reflex due to reduced venous return and subsequent ventricular contraction around a poorly filled cardiac chamber. Clonidine was about equiactive with ROP, but other DA agonists were not examined, with the result that direct comparative assessment cannot be made to current medications. These studies indicate that ROP, like bromocriptine and pergolide, would be expected to provoke orthostatic hypotension, particularly following initiation of treatment. Orthostatic hypotension that occurs with bromocriptine and pergolide is minimized by titration from a low initial dose, and similar administration should be employed with ROP. From studies in the cat, the hypotensive effect of ROP would be expected to be additive with that of L-DOPA. Specific studies were not performed to address the question, but a rebound increase in sympathetic release may be expected upon abrupt termination of ROP since this is a common property of inhibitors of neurotransmitter release.

Decreased urine flow, with concomitant reduction in potassium excretion was observed in concious rats, but the mechanism for this is unclear.

No significant effects other than those consistent with depression of sympathetic activity were noted on respiration or the autonomic nervous system.

**ADME/PK**

Oral dose absorption was very high in all species examined (94-100%). The volume of distribution was high in rat, monkey and human (2, 2.8 and 7.7 L/kg respectively) while protein binding was low (30, 10 and 11-25% respectively). Whole body radioautography and tissue sample quantitation in rats showed similar distribution for IV and oral dosing, with high levels in liver and kidney, consistent with high absorption and similar

routes of elimination. Brain levels after oral dosing of 2 mg/kg 14C-ROP were <MQL but analysis using IV administration indicated a tissue concentration about the same as heart and skeletal muscle, with a brain:blood ratio of about 1. Differences between brain regions were generally <2-fold (no attempt was made to delineate specific binding between regions which would be expected to show differences related to receptor density). A study using a 6 h infusion of 1 mg/kg 14C-ROP in monkey indicated a comparable distribution to rat, with brain levels 10-20% of kidney and liver. ROP had a higher penetration (4-fold) into rat brain compared to SKF 89124, but data were unclear for SKF 104557 due to the absence of a defined extraction efficiency. The ROP csf:plasma ratio was around 1, comparable to the brain:blood distribution. The equimolar partition across the blood brain/csf barriers is probably related to the positive logP for ROP (+2.4). The observation in rat that brain levels were below MQL following oral administration may have been due to low bioavailability of parent compound.

Bioavailability was limited by first-pass metabolism. This varied considerably between monkey and man, with the monkey having only 0.5-10% (dose-related) bioavailable compared to about 50% in humans for clinical doses. Since absorption was very high in all species, this indicates much less first pass metabolism in man.

ROP was extensively metabolized. Phase I metabolism was by hydroxylation to SKF 89124 or depropylation to SKF 104557 (with some subsequent metabolism). These metabolites were also present as glucuronides, especially SKF 89124. SKF 89124 was the primary metabolite in rat, comprising 50-60% of identified plasma compounds 1 h after administration of 1.75 mg/kg IV or 220 mg/kg p.o., with parent compound at 12%. Although SKF 89124 was pharmacologically active the receptor activity of glucuronidated SKF 89124 was not analysed. However, glucuronidation and low transfer into brain would be likely to limit the potential CNS activity of this metabolite. The levels of SKF 104557 varied in rat with values of 5, 28 and 31% of plasma radioactivity after single doses of 50, 150 and 220 mg/kg respectively in different studies. This may indicate some saturation of depropylation, as was observed in the monkey (see below), but the levels of ROP were not commensurately increased. SKF 104557 was the primary metabolite in mouse, monkey and man. The mouse, with about 40% of plasma radioactivity being SKF 104557, was similar to man (about 50%). In the monkey a number of additional metabolites were prominent, with SKF 104557 comprising about 17% of plasma compounds. Urinary metabolites were similar following IV or oral dosing in mouse, rat, monkey and man.

In the monkey metabolism was saturable, as indicated by a dose-dependent decrease in the proportion of parent compound metabolized, resulting in a much greater than dose proportional increase in ROP exposure with dose: the AUC increased 161 to 225-

fold from 1.5 to 15 mg/kg. However, increasing dose from 0.25-1.5 mg/kg produced somewhat less than a dose proportional increase in AUC (4.6-4.8 fold). Over the clinical dose range in humans there was approximately a linear pharmacokinetic profile following single or repeat doses, and no evidence of any saturation of metabolism.

was identified as the major enzyme involved using human liver microsomes (which showed much greater activity than kidney or lung), but ROP had little inhibitory on the enzyme activity due to low affinity, and ROP was only a weak inducer of CYP450 activity. Other ____ substrates would have the potential for altering the metabolism of ROP.

In rats treated for 14 days @50 mg/kg/day, exposure to the parent compound and the despropyl metabolite SKF 104557 increased as a proportion of dose (28.5% to 50.5% and 31.5 to 42.5% respectively while levels of the hydroxylated metabolite SKF 89124 decreased (47.5% to 6.5%). This suggests a down-regulation of metabolism rather than simple saturation. At the lower dose of 15 mg/kg/d, but not 1.5 mg/kg/d, there was some suggestion of the proportion of SKF 89124 decreasing, but levels of parent compound and SKF 104557 were too low for comparative analysis. In a 1 year monkey toxicity study (n=4 animals), exposure to ROP, and the relative exposure to SKF 104557, did not change consistently with time and dose, suggesting no real change in PK parameters. A 1 month study found an increase in ROP plasma levels with time for 5 and 15 mg/kg/d doses, but only one time point was examined and plasma levels on day 1 were close to MQL. Taken together, preclinical studies do not indicate that a change in PK parameters is likely following chronic clinical treatment with ROP.

The observed half life for ROP in rat, monkey and man was approximately 0.5 h, 1 h and 5-7 h respectively. Since a t.i.d. regimen gives a dosing interval close to the half life of ROP, the plasma level approximately doubled compared to single doses. The difference in pharmacokinetic parameters led to limited exposure ratios for ROP and metabolites in toxicological studies using mouse, rat and monkey such that maximum AUC ratios achieved in tox studies were 0.58, 2.8 and 0.92 relative to human daily exposure respectively. Cmax ratios were 11.7, 13 and 5-fold for mouse, rat and monkey respectively. Additional details are noted in the toxicology section below.

Excretion was primarily renal in all species, with urine of the rat and monkey containing approximately 60% and 70% of administered radiotracer and mouse and human containing about 90%. Fecal excretion accounted for much of residual excretion. With bile duct cannulation in rats, renal excretion was reduced to 23% and bile contained 76% of administered radioactivity, indicating entero-hepatic circulation.

**TOXICOLOGY**

Definitive studies were 1 year oral administration in the S-D rat (25/s/group) and cynomolgus monkey (4/s/gp).

In rats the HD was 100 mg/kg/d, which was selected due to 11/63 animals dying ● 125 mg/kg/d in a 6 month study, with a MD and LD of 50 and 5 mg/kg/d respectively. Clinical signs were prominent in the MD and HD groups, with stereotyped movements (hyperactivity, excessive grooming, sideways movements), ptosis and abnormal (hunched or low) posture occurring in more than 80% of animals. Additional observations were salivation, urine-wet fur, ocular discharge, Straub tail, aggression and alopecia. Convulsions were also observed in treated groups (after 6 weeks-1 year treatment), with 1, 3 and 8 animals exhibiting seizures in LD, MD and HD groups. In the HD group convulsions were associated with mortality in the majority of cases. Mortality overall was increased in the HD group (36%) and possibly the MD (12%) compared to controls (8%). The HD was therefore a MTD. Both M and F HD groups showed decreased body weight at study termination (about 13%) and an increase in food consumption of 13 and 27% respectively. Hyperactivity may account partly for these effects.

Prolactin levels were decreased in MD and HD males by ROP at study termination, with the HD group being about 6-fold lower than controls. 5 and 50 mg/kg/d produced no reduction and 62.5% inhibition respectively. Females showed no decrease in prolactin, possibly due to endogenous endocrine changes, but did exhibit an increased estrogen/progesterone ratio at 50 mg/kg/d and 100 mg/kg/d. In rats treated for 6 months prolactin levels were decreased at 10-125 mg/kg/d from 1 week through the whole study, suggesting prolactin would be decreased throughout both studies. However, the levels measured in the 1-year study were much greater than measured in the 6-month evaluation in Wistar rats. The assay methodology was essentially identical, so this difference may have been due to species difference and/or the timing of blood collection which was 24 hr post dose in the 12-month study and 2 hrs in the 6-month study. A number of toxicological findings in the 1 year rat study have been ascribed by the sponsor to hypoprolactinemia and other endocrine changes, with a model of interrelationships shown in Fig 36, including Leydig cell hyperplasia, enlarged ovaries with increased numbers of corpora lutea, changes in appearance of luteal cells, endometrial hyperplasia and vaginal cornification. Recovery was noted following 6-month treatment but was not examined in the longer study.

An increase in adrenal weight was observed in both M and F at 50 and 100 mg/kg/d (about 70% increase in relative weight) and was also observed in a 6-month study at 50 and 125 mg/kg/d. In the latter study a hyperplasia of the zona reticularis and zona

fasciculata of the cortical layer was noted. The sponsor notes that increased ratios of estrogen:progesterone are associated with increased ACTH release. Stress could also play a role, but similar changes were not observed at lower doses which should have an equivalent stress stimulus.

Relative liver weight increased approximately 25% in MD and HD F and also increased 18% in HD males. Hypertrophy was correspondingly noted in females (6/25, 3/25 and 1/25 at HD, MD and LD compared to none in controls). Hypertrophy was also observed in M+F in the HD group in a 6-month study and in F @ 50 mg/kg/d in the 2-year carcinogenicity study, with incidences of 25/70 in treated animals and 22/140 in controls. No changes in serum chemistry were noted in the 1 year study, but in a 6-month study ALT and AP showed about 50% increase at week 5, with scattered significant increases to the end of the study. Subacute studies also noted increases in these enzymes. These changes may be related to a moderate degree of P450 induction in both M and F (43-48%), with F being more sensitive. Females also showed a dose-related increase in hepatocellular alterations in specific foci (12/25 @ HD vs 3/25 in con) after 52 weeks treatment. These effects did not appear to increase the risk of tumor development.

A dose-related increase in gastric erosion or ulceration was noted after 1 yr treatment, and in some cases at high doses in subacute studies, but not in lifetime carcinogenicity studies.

A lesion observed only after 1.6-2 yr treatment in rats was mild-moderate retinal atrophy (and in some cases autolytic changes). This was not observed in controls but was present in all treated groups, with about equal gender sensitivity (4/137, 3/137 and 16/140 at 1.5, 15 and 50 mg/kg/d respectively for M+F combined). The sponsor notes that the majority of affected rats were housed at the highest light levels, suggesting an elevated light sensitivity. This toxicity is of concern because an increase was observed over control levels at 1.5 mg/kg/d, a dose that gives low exposure of parent and metabolites in the rat. Levels of ROP and SKF 104557 were generally not quantifiable (< 10 ng/ml) in rat following 1.5 mg/kg, but the AUC for SKF 89124 (which is a major metabolite in rat and only a minor metabolite in humans) was 9.7 ng.h/ml. This is less than half the exposure level of SKF 89124 in humans, indicating exposure to ROP and SKF 104557 was much lower. 50 mg/kg p.o. of ROP in rats gave an exposure 2.8 times higher than human for ROP itself and 2.2 times higher for SKF 104557. Since studies with $^{14}$C-ROP indicated approximately a proportional increase in plasma levels up to 50 mg/kg (single doses), exposure levels for 1.5 mg/kg/d p.o. could be anticipated to be 10 to 15-fold lower than in human. Additionally, it should be noted that in pigmented rats ROP was shown to bind to melanin, and melanin binding has been associated with retinal damage e.g. for chloroquine. Long term retention of

drug was demonstrated (half life about 20 days in eye) which could contribute to toxicity, and accumulation is also possible in pigmented tissue but was not analyzed. However, no drug related retinopathy was noted in rats or monkeys treated for 1 year at 100 or 15 mg/kg/d respectively.

The pivotal 1 year toxicity study in cynomolgus monkey employed doses of 1.5, 5 and 15 mg/kg/d p.o. The primary study submitted was the second study initiated, with the first being terminated after 4 weeks following 5 deaths in the HD group and several infections. The first study was inspected and the deaths, while probably partly due to infections, could not be completely dissociated from drug administration.

The monkey high dose was limited to 15 mg/kg/d due to excessive clinical signs and self mutilation at 30 mg/kg/d. Stereotyped behavior, excessive grooming and formication, probably the result of DA receptor agonism, was only observed at the 15 mg/kg dose. It is not clear whether this categorization included hyperactivity since the DSI investigation of the first study noted that HD animals were nearly in constant motion. Only HD M showed a decrease in body weight gain and no data on food consumption was provided. Prolactin levels generally were decreased in all dose groups, but due to high variability only HD M were significantly reduced. Relative adrenal weight was increased, as observed in rat, by 40%-50% in HD M and F (but not at lower doses). Brain weight increased in HD males only: no microscopic pathology was observed. Testis and epididymis weight was elevated in HD males without morphological abnormality. Ovary weight was increased in HD F and ascribed to ovarian cysts, which did not show a dose-related incidence. One case of retinal atrophy was observed in control and LD F groups, but preliminary signs were noted prior to treatment of the F monkey. Sporadic significant differences were observed in hematology parameters but these were not dose related or consistent.

These studies suggest that DA-receptor mediated toxicities are likely to be dose-limiting in humans, with hepatic enzyme alterations being signs of organ toxicity. Examination for retinal atrophy should be considered after prolonged clinical use.

**Carcinogenicity and genotoxicity**

Carcinogenicity studies employed single daily doses as compared to t.i.d. exposure in patients which, because of the short half life in rodents, resulted in a quite different pattern of exposure.

A 2-year carcinogenicity study in the mouse utilized doses of 5, 15 and 50 mg/kg/d. Clinical signs consistent with DA receptor

agonism were observed at the HD, although the HD produced less hyperactivity than was observed in a 60-day dose-ranging study. HD M had a decrease in body weight gain of 10-18% over the study compared to control groups while females were not different to controls at study termination. No increase in mortality was observed, with both controls and HD having about 40% survival. Biotransformation in the mouse was qualitatively similar to human, and in a 60-day study the HD produced a Cmax approximately 12-fold higher than human and an AUC 0.6 times human exposure. Increased exposure could probably have been achieved by dietary administration. The HD appears appropriately selected based on observed body weight changes and mortality observed in prior toxicology studies @ 100 mg/kg/d (1/20 in a 60-day study and 4/32 in a 90-day study). However, variability was observed between the dose ranging studies. The 2-year study suggests the 90-day study exhibited exaggerated mortality. An intermediate dose between 50 mg/kg/d and 100 mg/kg/d, or possibly higher with dietary administration, would have been a better selection, but repeating the study would be unlikely to provide a much improved risk assessment.

An increase in benign endometrial polyps was observed in HD F only. The sponsor considered this incidental, indicating that there was no difference to one control group and the incidence was within historical control values. However, historical data were not provided and the incidence was significantly different to combined controls. This effect should therefore be regarded as drug-related, especially as the HD was not necessarily the MTD and incidence may have risen with a higher dose. Hyperplasia of the urinary bladder epithelium was noted only in drug-treated animals (1F, 2M), but males only had small abnormal foci. Palpable masses were not increased.

Thus, only benign endometrial polyps were identified as drug-related in the mouse at the doses employed.

In the rat carcinogenicity study, doses of 1.5, 15 and 50 mg/kg/d were utilized. The HD was selected because of mortality at 100 mg/kg/d (36%) observed in previous studies. Biotransformation in the rat was somewhat different to the human, with hydroxylation to SKF 89124 being the predominant metabolism, although this metabolic route decreased on repeated drug exposure. Correspondingly, levels of SKF 89124 were much greater than in humans in a 14 day study (>10-fold higher AUC). Exposure to ROP and the primary human metabolite SKF 104557 were 2.8 and 2.2 times the human AUC respectively. Clinical signs due to DA receptor agonism were observed in nearly all HD animals, with an increased incidence of seizures, the majority of which were associated with preterminal mortality. The decrease in body weight gain for the HD group was -17%. The study was terminated early (100 weeks) to limit mortality, which was high in all

groups (about 20 survivors in each group, Figs 29 and 30). The question of statistical validity with low survival needs to be addressed. Apart from the number of surviving animals the HD was an appropriate MTD for single daily dose administration.

Leydig cell tumors and hyperplasia were increased in a dose-related manner, with the LD M only showing hyperplasia. 32/70 M had adenomas in the HD group, mainly accompanied by hyperplasia. Decreased prolactin levels (which were observed in rats treated for 6-months or 1 yr @ 50 mg/kg/d but not monitored in this study) from a variety of agents have been associated with increases in Leydig cell tumors. The mechanism of action has not been clearly delineated (Kovacevic et al., Int J. Andrology 10, 1987, 773-784), but appears to involve prolactin receptors on Leydig cells inducing down-regulation of LH receptors. Subsequent reduction in LH-dependent testosterone release elevates serum LH and induces a mitogenic response. A sponsor study indicated 50 mg/kg/d reduced rat Leydig cell LH receptor density, but serum LH levels were not affected in an 8-day study. A literature study indicated that serum LH levels do not increase until after about 4 weeks treatment. These data are therefore consistent with hypoprolactinemia leading to Leydig cell proliferation but are not definitive of such an effect. A lack of prolactin receptors on Leydig cells in humans may indicate a lack of cross-species predictability.

Benign skin fibromas were also increased (4/139 controls and 4/59, 7/60 and 7/70 in M LD, MD and HD groups). There was no increase in F. The sponsor considers this an incidental finding, noting the lack of effects in F and absence of related malignant tumors, but both MD and HD groups were affected and this should therefore be considered drug-related.

A single case of malignant uterine carcinoma occurred, in the HD F group. While not statistically significant a treatment-related effect is possible since malignant endometrial or myometrial tumors are associated with administration of other dopamine agonists, bromocriptine and pergolide. A difference in the incidence rate between DA agonists, if pharmacologically-mediated, could have been influenced by gavage administration of ROP compared to dietary dosing for bromocriptine and pergolide. The induction of uterine tumors has been ascribed to the increased estrogen/progesterone ratio induced by these dopamine agonists (resulting from prolactin inhibition), and a similar estrogen dominance was observed in 1 year studies in rats at doses of 50 and 100 mg/kg/d ROP. However, such endocrine effects are not observed in humans and thus the predictive value of these observations for human carcinogenicity risk is not known.

Hyperplastic foci were increased in the pituitary of MD and HD F, but this was associated with a decrease in tumor incidence,

suggesting reduced progression due to DA receptor inhibition of cellular activity. Although hypertrophy was observed in the liver, there was no significant increase in neoplasia.

Overall, in the rat, Leydig cell tumors were observed which may be dependent upon hypoprolactinemia. Skin fibromas were likely to be drug-related.

In vitro genotoxicity was examined in bacteria, L5178Y mouse lymphoma cells and human lymphocytes, and in vivo genotoxicity was studied in the mouse micronucleus test. ROP was not considered mutagenic in the Ames test up to 5000 μg/plate or in the mouse micronucleus test at 400 mg/kg p.o. ROP showed a weak mutagenicity in mouse lymphoma cells, with a small (2 to 3-fold) reproducible increase in mutation frequency at 5000 μg/ml and elevated frequency at 3000-4000 μg/ml. In one experiment 5000 μg/ml ROP gave a significant (2-3 fold) increase in clastogenic effects in human lymphocytes, but this was not reproducible and fell within the historical range for controls. ROP may therefore be considered weakly mutagenic at high doses in one assay.

**Reproductive Toxicology**

Studies of fertility, embryo-fetal toxicity and perinatal-postnatal toxicity were performed with rat, and the rabbit was utilized for the second embryo-fetal toxicity study. The rat was a poor choice for studies of a DA receptor agonist since prolactin, which is depressed by DA receptor agonists, is a key reproductive hormone in the rat. Initial studies using limited duration protocols for fertility, embryo-fetal toxicity and perinatal-postnatal toxicity showed that ROP treatment of females would reduce fertility above 10 mg/kg/d (administered 2 weeks prior to mating to E7), 40 mg/kg/d would induce abortion (administered E7-E16) and 30-40 mg/kg/d could produce reduced pup weight or postnatal mortality by reducing lactation (administered E16-P21). Several protocols were designed to use doses lower than these during sensitive periods in the F.

A male fertility study employing a high dose of 125 mg/kg/d (737 mg/m$^2$) showed a reduction in numbers of F pregnant at the HD, that was not significant, and no change in other fertility parameters. No changes in male parameters was noted. In F, fertility was examined in a protocol encompassing treatment for 2 weeks prior to mating with 5, 50 and 100 mg/kg/d; 5 mg/kg/d for E0-E8; and then a repeat of the initial dose for E9-E20. Under this protocol clinical signs were observed in the dams, while postimplantation loss was reduced and the number of live fetuses increased at the HD. ROP therefore, within the limitations of studies in rats, did not reduce fertility at 125 mg/kg in M and

100 mg/kg in F.

In the definitive rat teratology study ROP administration was limited to 20 mg/kg/d for days E6 and E7 of pregnancy to avoid abortion. From E8 to E15 a dose range of 20-150 mg/kg/d was employed. No significant changes occurred in implantation or live births although postimplantation loss increased from 15% in controls to 22% in the HD, mean fetal weight was reduced at 120 and 150 mg/kg/d by 8-10%, and metatarsals had decreased ossification in MD and HD groups. The sponsor concludes that these were related to maternal toxicity. Digit-related malformations were observed in 1 litter which were probably related to ROP since adactyly and aphalangy were observed in a preliminary study in 6/27 litters from F treated with 30 mg/kg/d followed by 150 mg/kg/d. Two other litters (4 fetuses) had cardiovascular and neural tube defects, which were not consistently observed or dose related within or between studies. In the preliminary study, the day on which the dose increased from 30 mg/kg was varied between groups, with the group treated for days E7-10 @ 30 mg/kg/d and days E11-16 @ 150 mg/kg/d being most sensitive to digit malformations (3/10 litters affected). Studies subsequent to the definitive teratology study were interpreted by the sponsor to indicate that ROP was most teratogenic on E10, and that digit malformations were only seen at 150 mg/kg/d. However, the data are also consistent with an effect of 30 mg/kg/d ROP over days E6 and E7. Exposure data are not available for these doses, but 50 mg/kg/d produced a ROP AUC 2.8-fold higher than in patients and a Cmax 13-fold higher. One possible mechanism for digit abnormalities is vasodilation in the autopod and rupture of vessels, followed by hypoxia, since the sponsor notes a similarity between digit malformations in these studies and teratogenicity following uterine vascular clamping.

Therefore, although effects varied with the days of exposure, ROP was teratogenic in the rat at 150 mg/kg, with a possible influence of 30 mg/kg on specific days of pregnancy.

In preliminary rabbit teratology studies, postimplantation deaths were nearly twice the control rate at 25 mg/kg/d (275 $mg/m^2/d$; Cmax about 10-fold higher than patient Cmax), although no further increase with dose was noted. The definitive embryo-fetal toxicity study used a HD of 20 mg/kg/d, which produced clinical signs and 10% mortality of does. No drug-related teratogenic effects were apparent.

An embryo-fetal toxicity study in the rabbit employed 10 mg/kg/d ROP and 250 mg/kg L-DOPA alone and in combination. The combination produced exaggerated clinical signs consistent with additive dopaminergic activity in the does and mortality (3/20

does). L-DOPA alone produced malformations, primarily of digits, with some sternebrae abnormalities. The combination group did not have a higher total incidence of litters with abnormalities, but the number of affected fetuses was increased. Furthermore, adactyly, which was not observed in controls, occurred in 3/15 litters, with 10/137 fetuses affected, in the group receiving combination treatment. In contrast to the conclusion of the sponsor, this suggests that ROP and L-DOPA exert synergistic teratogenic effects.

In a perinatal-postnatal toxicity study in rats, pregnant females showed mild clinical signs at a HD of 10 mg/kg/d. At weaning the pups had decreased body weight (15-20%), but were similar to controls after 8 weeks, suggesting decreased prolactin may have affected lactation. A small delay in development that was observed may have been due to the lower weight of treated-group pups. A gender specific decrease in startle reflex in F, treated at 1 or 10 mg/kg, at 1 month and in the HD group at 2 months was noted, although other tests of neurological development were normal. Since, in a preliminary study using 20 mg/kg, startle reflex deficits did not correlate with fetal body weight, this neurological toxicity may not have been related to lactation inhibition. No effects were noted on $F_1$ generation reproductive function.

**Labeling**



The following sections are excerpted from the sponsor's suggested labeling for ROP (pages indicated in parentheses refer to sponsor's annotated labeling, volume 2, item 2, section 2A): Redline ([redacted]) indicates sections with suggested alterations, which are indicated in **bold**.

CLINICAL PHARMACOLOGY
Pharmacodynamics



Studies with human cloned receptors *in vitro* show that *Requip* binds with high affinity to cloned human [redacted] receptors. (page 20)



The anti-Parkinson activity of *Requip* is believed to be due to its potent stimulatory effects on post-synaptic dopamine [redacted] receptors within the caudate-putamen in the brain. (page 20)

Pharmacokinetics

The N-despropyl metabolite is the major metabolite circulating in plasma; however, there is no evidence of any saturation of its formation after single or repeat oral administration of

[redacted]. Systemic plasma concentrations of the hydroxylated metabolite are low and account for about 1 to 5% of the [redacted] concentrations. [redacted].
(page 34)

PRECAUTIONS

Carcinogenesis, Mutagenesis, Impairment of Fertility

Two-year carcinogencity studies were conducted in the mouse and rat at dosages up to 50 mg/kg [redacted]. In the rat, drug-induced interstitial (Leydig) cell hyperplasia and/or adenoma were observed in the testes at all doses tested, i.e., ≥1.5 mg/kg [redacted].
(page 49)

*Requip* did not cause gene mutation or chromosome damage in [redacted] genotoxicity assays, including the bacterial mutagenicity tests (*Salmonella typhimurium and Escherichia coli*) *in vitro* chromosome aberration test in human lymphocytes, [redacted] and *in vivo* mouse micronucleus test.
(page 50)

At doses >10 mg/kg [redacted], *Requip* caused disruption of implantation in the pregnant rat due to the prolactin-lowering effect of *Requip*. [redacted]. In specially designed studies in the rat using [redacted] doses during prolactin-dependent phases of pregnancy and lactation, *Requip* did not affect female fertility at dosages up to 100 mg/kg [redacted]. No effect on male fertility was observed in rats employing dosages up to 125 mg/kg [redacted].
(page 51)

Pregnancy

Teratogenic effects- Pregnancy Category C:
*Requip* given to pregnant rats during organogenesis resulted in decreased fetal body weight at 60 mg/kg [redacted],

increased fetal death at 90 mg/kg [redacted] and digital malformations at 150 mg/kg [redacted]. These was no indication of an effect on development of the conceptus at a maternally toxic dose of 20 mg/kg [redacted] in the rabbit. There are no adequate and well-controlled studies in pregnant women. The use of *Requip* during pregnancy is not recommended.

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Suggested revisions:

CLINICAL PHARMACOLOGY
Pharmacodynamics

Replace the terms "$D_2$, $D_3$, $D_{4.4}$" in paragaph 1 and "$D_2$" of paragraph 2 with "$D_2$-type"

Insert:
**"Requip binds to melanin-containing tissues (e.g. eye) to a greater degree than non-pigmented tissues, and tissue levels decline with a half life of 16-20 days."**

Pharmacokinetics

Revise paragraph to read:
The N-despropyl metabolite is the major metabolite circulating in plasma; however, there is no evidence of any saturation of its formation after single or repeat oral administration of ***Requip***. Systemic plasma concentrations of the hydroxylated metabolite account for about 1 to 5% of the ***Requip*** concentrations. **The N-despropyl metabolite has low binding affinity for dopamine receptors. The hydroxylated metabolite has a higher affinity than ropinirole for $D_2$-type receptors but, because of low systemic concentrations, is unlikely to exhibit significant pharmacological activity in humans.**

PRECAUTIONS

Carcinogenesis, Mutagenesis, Impairment of Fertility

Two-year carcinogencity studies were conducted in the mouse and rat at dosages up to 50 mg/kg **(equivalent to 150 mg/m² in mice and 295 mg/m² in rats). On a mg/m² basis these doses exceeded the maximum recommended human dose (MRHD) by 10-fold in mice and 20-fold in rats. However, in short-term studies the relative daily systemic exposure, compared to humans receiving the maximum**

recommended dose, for ropinirole was 0.6 in the mouse and 2.8 in the rat. In the rat, drug-induced interstitial (Leydig) cell hyperplasia and/or adenoma were observed in the testes at all doses tested, i.e., ≥1.5 mg/kg (0.6 times MRHD on a mg/m² basis). These lesions are believed to be related to Requip-induced reduction of serum prolactin. Since rat, but not human, Leydig cells possess prolactin receptors, the relevance to humans is not known. The incidence of benign skin fibromas was also elevated. In the mouse study Requip induced an increased incidence of uterine endometrial polyps at 50 mg/kg.

*Requip* did not cause gene mutation or chromosome damage in several genotoxicity assays, including the bacterial mutagenicity tests (*Salmonella typhimurium and Escherichia coli*), *in vitro* chromosome aberration test in human lymphocytes and the *in vivo* mouse micronucleus test. In an *in vitro* mouse lymphoma (L5178Y cells) mutagenicity assay, an increased mutation frequency was observed only at the highest recommended concentration (5 mg/ml), following activation by rat liver microsomes. The relevance of this observation to humans is unknown.

At doses >10 mg/kg (>59 mg/m², >4 times MRHD), *Requip* caused disruption of implantation in the pregnant rat. This effect is thought to be due to the prolactin-lowering effect of *Requip*. In humans, chorionic gonadotropin, not prolactin, is essential for implantation. In specially designed studies in the rat using low doses during prolactin-dependent phases of pregnancy and lactation, *Requip* did not affect female fertility at dosages up to 100 mg/kg (590 mg/m², 40 times MRHD). No effect on male fertility was observed in rats employing dosages up to 125 mg/kg (737 mg/m², 50 times MRHD).

Pregnancy

Teratogenic effects- Pregnancy Category C:
*Requip* given to pregnant rats during organogenesis resulted in decreased fetal body weight at 60 mg/kg (354 mg/m², 24 times MRHD), increased fetal death at 90 mg/kg (531 mg/m², 36 times MRHD) and digital malformations at 150 mg/kg (885 mg/m², 60 times MRHD). These was no indication of an effect on development of the conceptus at a maternally toxic dose of 20 mg/kg (220 mg/m², 15 times MRHD) in the rabbit. In a teratogenicity study employing oral doses of *Requip* 10 mg/kg (110 mg/m²) and L-DOPA 250 mg/kg (2750 mg/m²) in the rabbit, combination treatment resulted in

**teratogenic effects (adactyly) not observed in controls or following either drug alone. *Requip* at 10 mg/kg (59 mg/m², 4 times MRHD) impaired growth and development of nursing offspring in a perinatal-postnatal study in rats. A reduction in lactation could underlie this effect. Female offspring of rats treated with 10 mg/kg showed altered neurological development. There are no adequate and well-controlled studies in pregnant women. The use of *Requip* during pregnancy is not recommended.**

**Recommendations**

The studies submitted adequately describe the pharmacological and toxicological characteristics of Requip (ropinirole HCl) and support approval for symptomatic treatment of Parkinson's disease.

Proposed labelling changes are detailed above.

B. Ault 11/14/96

Brian Ault

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HFD-120 R. Nighswander
HFD-120 B. Ault

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