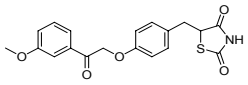
# mTOT-modulating insulin sensitizer, in Sprague Dawley (SD) and Long-Evans (LE) rats

Wade J. Adams[1], Jerry R. Colca[1], Alfred W. Lordi[2], and Eric G. Solon[2]
[1]Metabolic Solutions Development Company, Kalamazoo, MI 49007; [2]QPS, LLC, Newark, DE 19711

## BACKGROUND

- MSDC-0602 (5-[[4-[2-(3-methoxyphenyl)-2-oxoethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione) is an mTOT insulin sensitizer being developed for the treatment of Type 2 diabetes.
- The compound exerts its pharmacology by selectively modulating mitochondrial metabolism resulting, among other things, in improved insulin sensitivity and an increase in brown adipose tissue.
- MSDC-0602 is related to the thiazolidinedione pioglitazone (ACTOS™) but has significantly reduced ability to activate the nuclear transcription factor PPARγ.



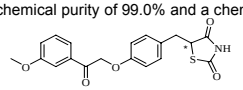
MSDC-0602

## OBJECTIVES

- The objectives of this study in male Sprague Dawley (SD) and Long-Evans (LE) rats were to determine the mass balance excretion, plasma pharmacokinetics (PK), and tissue distribution patterns of [$^{14}$C]MSDC-0602-related material following a single 10 mg/kg oral (PO) administration of [$^{14}$C]MSDC-0602.
- The study was also conducted to provide an estimation of the human $^{14}$C radiation dosimetry and to provide plasma, bile, and excreta for metabolite profiling and identification analyses.

## IN-LIFE STUDY DESIGN

- Five groups of male SD rats and one group of male LE rats (28 total rats; see table below), approximately 7 weeks of age and weighing 244 g to 277 g at the time of dosing, were administered a single oral bolus dose of [$^{14}$C]MSDC-0602 at a target dose of 10 mg/kg (50 µCi/kg).
- [$^{14}$C]MSDC-0602 ([thiazolidinedione-5-$^{14}$C]MSDC-0602) had a specific activity of 55 mCi/mmol, radiochemical purity of 99.0% and a chemical purity 98.4%.



- [$^{14}$C]MSDC-0602 was diluted with MSDC-0602 (98.5% chemical purity)
- The formulation contained 1% (w/w) carboxymethylcellulose, sodium salt, 0.01% (w/w) Tween 80 in deionized water solution as the dosing vehicle that contained [$^{14}$C]MSDC-0602 at a final concentration of 1.01 mg/mL, and 3.89 µCi/g.

| Group | Strain | N | Purpose | Dose Route | Dose Vol. (mL/kg) | Target Dose (mg/kg) | Target Radioactivity Level (µCi/kg) |
|---|---|---|---|---|---|---|---|
| 1 (Intact) | SD | 3 | Mass Balance | Oral | 10 | 10 | 50 |
| 2 (Intact) | SD | 3 | Expired Air | Oral | 10 | 10 | 50 |
| 3 (BDC) | SD | 4 | Biliary Excretion | Oral | 10 | 10 | 50 |
| 4 (JVC) | SD | 5 | PK | Oral | 10 | 10 | 50 |
| 5 (Intact) | SD | 3 | QWBA | Oral | 10 | 10 | 50 |
| 6 (Intact) | LE | 10 | QWBA | Oral | 10 | 10 | 50 |

BDC: bile duct cannulated
JVC: jugular vein cannulated
SD: Sprague Dawley
LE: Long-Evans

- Animals in Groups 1 (SD) and 3 (SD) were used to determine mass balance, and had excreta collected and analyzed by liquid scintillation counting (LSC). Group 3 animals were bile duct-cannulated (BDC). Samples were collected from Group 1 animals to 192 h, and from Group 3 animals to 72 h
- Animals in Group 2 (SD), which were used to determine radioactivity in expired air (pre-dose to 72 h), were placed into individual closed glass expired air collection chambers following dosing.
- Each animal in Group 4 (SD) provided serial blood samples (pre-dose to 72 h) for plasma pharmacokinetic (PK) determination. Animals in Group 4 were jugular vein cannulated (JVC).
- Animals in Groups 5 (SD) and 6 (LE) were for tissue distribution determinations, and a terminal blood sample (~3 mL) was obtained via cardiac puncture.

## IN-LIFE STUDY DESIGN (continued)

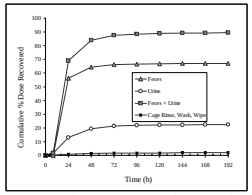
- All rats from Group 1 were euthanized at 192 h post-dose. All rats from Group 2, 3, and 4 were euthanized at 72 h post-dose. One rat per time point from Group 5 was euthanized at 2 h, 24 h, and 168 h post-dose. One rat per time point from Group 6 was euthanized at 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 144 h, and 192 h post-dose.
- Aliquots of blood and homogenized feces samples were combusted (PerkinElmer Model 307 Sample Oxidizer) prior to LSC. Feces samples were homogenized with approximately 3 volumes of water:methanol (1:1).

## RESULTS

### Excretion of Radioactivity by Intact SD Rats in Expired Air Following PO Administration of [$^{14}$C]MSDC-0602

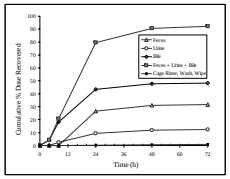
- Recovery of radioactivity in expired air accounted for < 0.1% of the administered dose through 72 h post-dose.

### Mean Time Course of Excretion of Radioactivity by Intact SD Rats Following PO Administration of [$^{14}$C]MSDC-0602



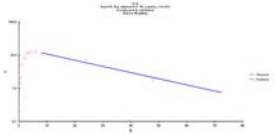

- Feces was the primary route of excretion (67%); > 56% of the dose was excreted in first 24 h.
- A mean of 22.4% of the dose was excreted in urine.
- A total of 91.5% of the dose was recovered

### Mean Time Course of Excretion of Radioactivity by Bile Duct-Cannulated SD Rats Following PO Administration of [$^{14}$C]MSDC-0602




- Bile was the primary route of excretion in BDC rats, with a mean of 48.1% of the dose excreted in bile.
- A mean of 31.7% of the dose was excreted in feces.
- A mean of 12.3% of dose was excreted in urine
- A mean of 92.8% of the total dose was recovered; the majority of radioactivity was excreted in the first 24 h post-dose.
- Total recovery in urine and bile (60.4%) suggested that the extent of oral absorption was at least 60%.

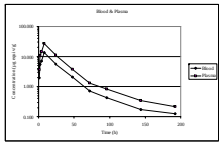
### Typical Plama Concentration-Time Curve in Intact JVC Male SD Rats Following PO Administration of [$^{14}$C]MSDC-0602



- Mean plasma $C_{max}$ observed in intact male jugular vein cannulated SD rats was 15.280 µg equiv/mL at 8 h post-dose.
- Plasma radioactivity concentrations decreased from $C_{max}$ with an arithmetic mean (±SD) apparent terminal disposition half-life of 14.7 h (range – 13.1 to 16.2 h).
- Plasma total radioactivity $AUC_{last}$ calculated from mean concentration data was 432.125 µg equiv•h/mL.

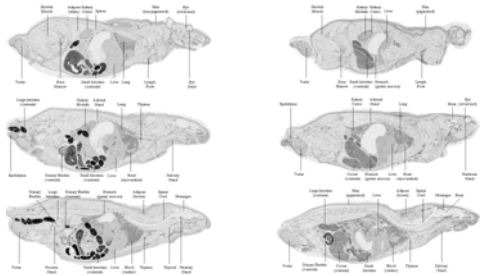
## RESULTS

### Blood and Plasma Concentration-Time Data in Male LE Rats

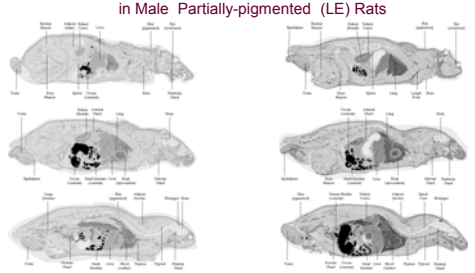



- Blood to plasma ratios data indicated little or no partition into red blood cells.

### Autoradiograms of [$^{14}$C]MSDC-0602 24 h Post-dose in Male Albino (SD; Left) and Male Partially-pigmented (LE; Right) Rats



### Autoradiograms of [$^{14}$C]MSDC-0602 4 h (Left) and 8 h (Right) Post-dose in Male Partially-pigmented (LE) Rats



### QWBA of [$^{14}$C]MSDC-0602 in Albino (SD) Rats

- [$^{14}$C]MSDC-0602-derived radioactivity was absorbed, widely distributed, and most tissues had concentrations that were lower than blood from 2 h through 24 h post-dose.
- [$^{14}$C]MSDC-0602-derived radioactivity in most tissues (24 of 40 tissues) were found at 2 h post-dose, the first of three time points collected.
- Thirteen of 40 tissues reached $C_{max}$ at 24 h post-dose and all tissues were below quantifiable limits (BQL) at 168 h post-dose.
- The highest overall concentrations were observed in the contents of the alimentary canal, and in bile and urine.

### QWBA of [$^{14}$C]MSDC-0602 in Partially-pigmented (LE) Rats

- [$^{14}$C]MSDC-0602-derived radioactivity was absorbed, widely distributed, and most tissues had concentrations that were lower than blood through 192 h.
- $C_{max}$ of [14C]MSDC-0602-derived radioactivity in most tissues were found at 8 h post-dose.
- [$^{14}$C]MSDC-0602-derived radioactivity concentrations > 10 µg equiv/g at Cmax were found in small intestine, cecum, meninges, blood, lymph nodes, oral mucosa, kidney cortex, urinary bladder and lung.
- The highest overall concentrations were observed in the contents of the alimentary canal, and in bile and urine; tissues began to exhibit decreased concentrations at 24 h post-dose.
- Elimination of radioactivity was not complete at 192 h post-dose, but most tissues were BQL except for blood, lung and kidney cortex, which had levels < 2% of Cmax.

## CONCLUSIONS

- Recovery of radioactivity in expired air accounted for less than 0.1% of the administered [$^{14}$C]MSDC-0602 dose through 72 h post-dose, confirming stability of the radiolabel.
- In intact SD rats, excretion of [$^{14}$C]MSDC-0602-derived radioactivity in feces (67.0%) and urine (22.4%) was rapid, the majority of radioactivity being eliminated in the initial 24 h post-dose; total recovery of radioactivity was 91.5%.
- In bile duct-cannulated SD rats, excretion of [$^{14}$C]MSDC-0602-derived radioactivity in bile (48.1%), feces (31.7%) and urine (12.3%) was rapid, the majority of radioactivity being eliminated in the initial 24 h post-dose; total recovery of radioactivity was 92.8%.
- Total recovery of radioactivity in bile (48.1%) and urine (12.3%) in BDC SD rats suggested that the extent of oral absorption was 60%.
- Radioactivity recovered in urine from intact rats (22.4%) was higher than in BDC rats (12.3%), and total radioactivity recovered in feces and bile was higher in BDC rats (79.8%) than in the feces of intact rats (67.0%), suggesting enterohepatic recirculation of radioactivity.
- Plasma radioactivity concentration profiles in JVC SD rats achieved maximum levels 8 h post-dose and then declined with an arithmetic mean terminal disposition half-life of 14.7 h.
- In albino (SD) rats, [$^{14}$C]MSDC-0602-derived radioactivity was absorbed, widely distributed, and most tissues had concentrations that were lower than blood from 2 h through 24 h post-dose.
- $C_{max}$ of [$^{14}$C]MSDC-0602-derived radioactivity in most tissues (24 of 40 tissues) were found at 2 h post-dose in albino rats, the first of 3 time points measured (2, 24, and 168 h), indicating rapid absorption.
- In albino rats, the highest overall concentrations of radioactivity were observed in the contents of the alimentary canal, and in bile and urine, which reflect the routes of elimination of [$^{14}$C]MSDC-0602-derived radioactivity and the normal movement of a radioactive dose after oral dosing.
- In partially-pigmented (LE) rats, [$^{14}$C]MSDC-0602-derived radioactivity was absorbed, widely distributed, and most tissues had concentrations that were lower than blood through 192 h post-dose.
- $C_{max}$ of [$^{14}$C]MSDC-0602-derived radioactivity in most tissues (31 of 41 tissues) were found at 8 h post-dose, indicating rapid distribution.
- The highest overall concentrations of [$^{14}$C]MSDC-0602-derived radioactivity in partially-pigmented rats were observed in the contents of the alimentary canal, and in the bile and urine, which reflect the routes of elimination of [$^{14}$C]MSDC-0602-derived radioactivity and normal movement of a radioactive dose after oral dosing.
- Radioactive concentrations observed in melanin-containing tissues in partially-pigmented rats indicated that there was an association of [$^{14}$C]MSDC-0602-derived radioactivity with melanin, but the association was reversible and radioactivity in pigmented tissues were BQL by 144 h post-dose.
- There was little or no partitioning of [$^{14}$C]MSDC-0602-related radioactivity into red blood cells.

## OVERALL CONCLUSIONS

- MSDC-0602, a novel mTOT-modulating insulin sensitizer, was confirmed to be predominantly excreted in the bile with enterohepatic recirculation of radioactivity suggested; the mean terminal plasma half-life was 14.7 h.
- MSDC-0602 tissue distribution was extensive and diverse in both non-pigmented and partially-pigmented rats; there was an association of radioactivity with melanin but the association was reversible.
- The results of this study represent part of the ADME package for the continued development of this novel anti-diabetic agent.