FINAL REPORT

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ADME Tox

Study of Etifoxine and GRX-917

STUDY NUMBER
100020843

Jun TANG, Ph.D.

March 31, 2015

CONFIDENTIAL

1. STUDY REFERENCES

Study title	**ADME Tox** **Study of ETX and GRX-917**	
Study number	**100020843**	**FINAL REPORT** **March 31, 2015**
Experimental period	March 5, 2015 - March 27, 2015	
PO number	03012015	

2. PERSONS INVOLVED IN THE STUDY

Investigator	**Eurofins Panlabs** 15318 NE 95th Street Redmond, WA 98052 U.S.A. Tel: +(001) 425 895 8666 Fax: +(001) 425 895 8668	**Jun TANG, Ph.D.** Principal Scientist, Drug Metabolism and Pharmacokinetics Tel: +1 425 284 3282 Fax: +(001) 425 895 8668
Study sponsor	**ANVYL LLC** 18 Technology Dr. IRVINE, CA 92618 U.S.A.	**Olivier DASSE**

3. APPROVAL

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I certify that this report accurately reflects all relevant data collected in this study.

Date March 31, 2015

Signature

Quality assurance statement

Eurofins Panlabs's Quality Unit certifies that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the raw data.

Date March 31, 2015

Signature

Gerry Evans

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5. PURPOSE OF THE STUDY

The purpose of this study was to test ETX and GRX-917 in *in vitro* metabolism assays.

6. COMPOUNDS

6.1. Test Compounds

Manufacturer: **ANVYL LLC**

Client Compound ID	Compound ID	Reference Number	Batch Number	FW	MW	Purity	Received Form	Stock solution	Flag
ETX	100020843-1	-	-	300.79	300.79	-	Powder	1.E-02 M DMSO	-
GRX-917	100020843-2	-	-	305.82	305.82	-	Resin	1.E-02 M DMSO	-

FW: Formula Weight - MW: Molecular Weight

6.2. Reference Compounds

In each experiment and if applicable, the respective reference compounds were tested concurrently with ETX and GRX-917, and the data were compared with historical values determined at Eurofins. The experiment was accepted in accordance with Eurofins validation Standard Operating Procedure.

7. RESULTS

7.1. ADME-Tox: *In Vitro* Metabolism

7.1.1. Test Compound Results

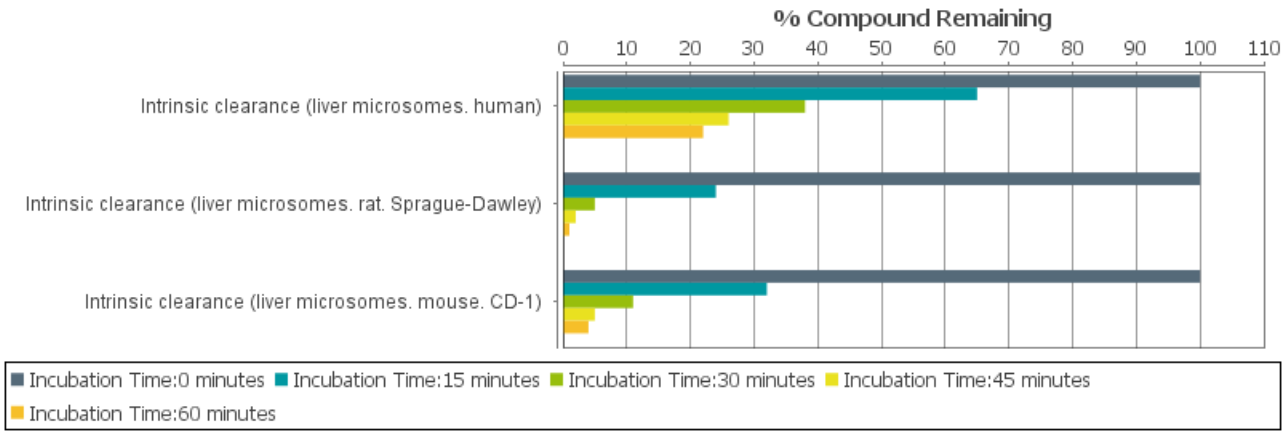

Figure 1. Histogram for ETX

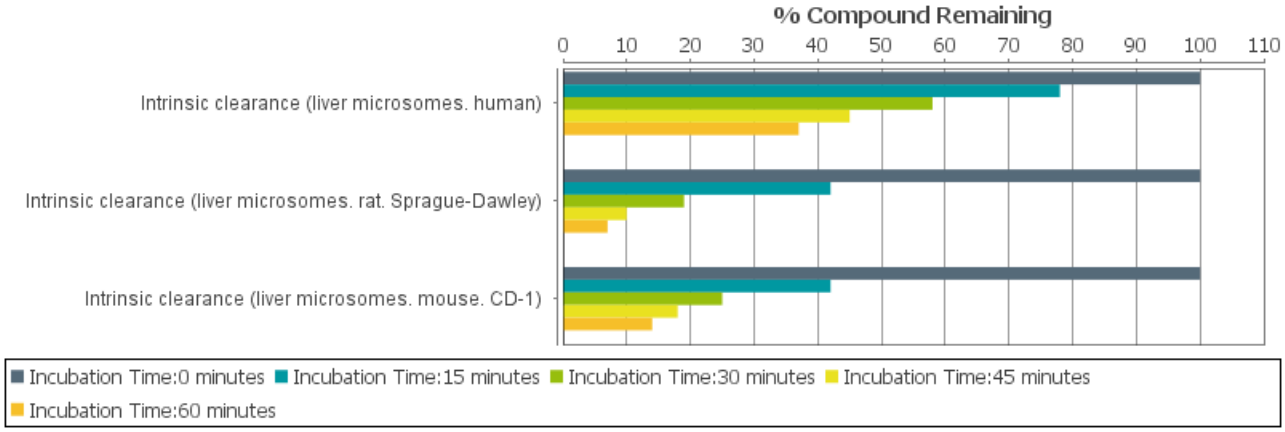

Figure 2. Histogram for GRX-917

Compound I.D.	Client Compound I.D.	Test Concentration	Incubation Time(minutes)	% Compound Remaining			Half-Life (minute)			Clint
				1st	2nd	Mean	1st	2nd	Mean	
Intrinsic clearance (liver microsomes, human)										
100020843-1	ETX	1.0E-07 M	0	100.0	100.0	100	23.0	21.9	22	308.7
100020843-1	ETX	1.0E-07 M	15	66.9	63.7	65				
100020843-1	ETX	1.0E-07 M	30	40.5	35.0	38				
100020843-1	ETX	1.0E-07 M	45	26.2	25.1	26				
100020843-1	ETX	1.0E-07 M	60	24.0	20.2	22				
100020843-2	GRX-917	1.0E-07 M	0	100.0	100.0	100	39.7	41.8	41	170.2
100020843-2	GRX-917	1.0E-07 M	15	74.4	81.0	78				
100020843-2	GRX-917	1.0E-07 M	30	52.9	63.5	58				
100020843-2	GRX-917	1.0E-07 M	45	42.1	48.4	45				
100020843-2	GRX-917	1.0E-07 M	60	35.8	37.3	37				
Intrinsic clearance (liver microsomes, rat, Sprague-Dawley)										
100020843-1	ETX	1.0E-07 M	0	100.0	100.0	100	7.7	7.4	8	915.1
100020843-1	ETX	1.0E-07 M	15	23.4	24.7	24				
100020843-1	ETX	1.0E-07 M	30	5.6	5.3	5				
100020843-1	ETX	1.0E-07 M	45	1.8	1.6	2				
100020843-1	ETX	1.0E-07 M	60	1.1	0.8	1				
100020843-2	GRX-917	1.0E-07 M	0	100.0	100.0	100	13.8	13.8	14	503.3
100020843-2	GRX-917	1.0E-07 M	15	42.6	41.8	42				
100020843-2	GRX-917	1.0E-07 M	30	20.2	18.4	19				
100020843-2	GRX-917	1.0E-07 M	45	10.4	10.6	10				
100020843-2	GRX-917	1.0E-07 M	60	6.4	6.8	7				
Intrinsic clearance (liver microsomes, mouse, CD-1)										
100020843-1	ETX	1.0E-07 M	0	100.0	100.0	100	10.5	10.6	11	656.9
100020843-1	ETX	1.0E-07 M	15	31.0	33.1	32				
100020843-1	ETX	1.0E-07 M	30	10.3	11.7	11				
100020843-1	ETX	1.0E-07 M	45	5.3	5.4	5				
100020843-1	ETX	1.0E-07 M	60	3.5	3.5	4				
100020843-2	GRX-917	1.0E-07 M	0	100.0	100.0	100	14.5	15.4	15	463.5
100020843-2	GRX-9171	1.0E-07 M	15	39.3	44.0	42				
100020843-2	GRX-917	1.0E-07 M	30	23.8	26.0	25				
100020843-2	GRX-917	1.0E-07 M	45	17.3	18.6	18				
100020843-2	GRX-917	1.0E-07 M	60	12.7	14.4	14				

7.1.2. Reference Compound Results

Compound I.D.	Test Concentration	Half-Life (minute)			Clint
		1st	2nd	Mean	
Intrinsic clearance (liver microsomes, human)					
imipramine	1.0E-07 M	>60	>60	>60	<115.5
propranolol	1.0E-07 M	162.7	136.0	>60	<115.5
terfenadine	1.0E-07 M	8.9	7.2	8	872.4
verapamil	1.0E-07 M	19.8	19.8	20	350.6
Intrinsic clearance (liver microsomes, rat, Sprague-Dawley)					
imipramine	1.0E-07 M	4.9	4.8	5	1442.2
propranolol	1.0E-07 M	3.3	2.6	3	2384.8
terfenadine	1.0E-07 M	7.6	7.3	7	931.8
verapamil	1.0E-07 M	24.9	23.9	24	284.6
Intrinsic clearance (liver microsomes, mouse, CD-1)					
imipramine	1.0E-07 M	15.1	15.4	15	453.4
propranolol	1.0E-07 M	13.6	12.9	13	522.7
terfenadine	1.0E-07 M	10.5	11.2	11	637.8
verapamil	1.0E-07 M	18.0	17.3	18	392.8

Note: Unit of Clint is µL/min/mg for microsomes, S9 and UGT assays; µL/min/pmol for CYP assays; µL/min/Million cells for hepatocyte assays

8. MATERIALS AND METHODS

8.1. Experimental Conditions

8.1.1. ADME-Tox: *In Vitro* Metabolism

Assay	Source	Substrate	Incubation	Measured Component	Detection Method	Bibl.
ADME						
Intrinsic clearance (liver microsomes, human)	Human liver microsomes (0.1 mg/mL)	Test compound	0, 15, 30, 45, 60 min 37°C	Test compound	HPLC-MS/MS	828
Intrinsic clearance (liver microsomes, rat, Sprague-Dawley)	Rat liver microsomes (0.1 mg/mL)	Test compound	0, 15, 30, 45, 60 min 37°C	Test compound	HPLC-MS/MS	828
Intrinsic clearance (liver microsomes, mouse, CD-1)	Mouse liver microsomes (0.1 mg/mL)	Test compound	0, 15, 30, 45, 60 min 37°C	Test compound	HPLC-MS/MS	828

8.2. Analysis and expression of results

8.2.1. ADME-Tox: *In Vitro* Metabolism

Intrinsic Clearance (microsomes, S9, cryopreserved hepatocytes, recombinant CYP, recombinant UGT)

Metabolic stability, expressed as percent of the parent compound remaining, was calculated by comparing the peak area of the compound at the time point relative to that at time-0. The half-life ($T_{1/2}$) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) vs. time, assuming the first-order kinetics. The apparent intrinsic clearance (CL_{int}, in µL/min/pmol, µL/min/mg or µL/min/Mcell) was calculated according to the following formula:

$$CL_{int} = \frac{0.693}{T_{1/2} * (\text{mg protein/µL or million cells/µL or pmol CYP isoyme/µL})}$$

9. BIBLIOGRAPHY

828. Obach, R.S. et al. (1997), *J. Pharmacol. Exp. Ther.,* 283 *: 46-58.*