

## ARTICLE IN REVIEW:

# TruVivo® Enables Improved Aldehyde Oxidase Clearance Predictions

### PUBLICATION: *Drug Metabolism and Disposition*, 2025

**TITLE:** Improved clearance predictions for aldehyde oxidase substrates using a novel triculture human hepatocyte model<sup>1</sup>

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**STUDY DESIGN:** Preclinical (*in vitro*)

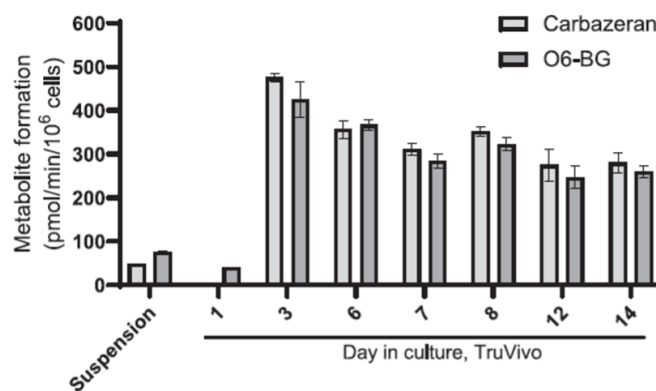
**SUMMARY:** Efforts to reduce CYP-mediated metabolism in drug development have increased reliance on alternative pathways such as aldehyde oxidase (AO), creating challenges for accurate clearance predictions. This study evaluated TruVivo, a human hepatocyte triculture system, for its ability to maintain physiologically relevant AO activity and improve *in vitro-in vivo* correlation (IVIVC) for AO substrates. AO activity in TruVivo was compared with suspended hepatocytes, plated monocultures, and HepatoPac cocultures (BioIVT), and its stability was monitored over 14 days.

Results showed that TruVivo sustained high and stable AO activity for at least 14 days with up to 10-fold higher activity than HepatoPac and approximately more than double that of plated monocultures. Researchers evaluated five aldehyde oxidase (AO) substrates—carbazeran, zoniporide, zaleplon, O6-benzylguanine (O6-BG), and XK-469—to predict hepatic clearance ( $CL_h$ ) and estimate the fraction metabolized by AO ( $f_{m,AO}$ ).  $CL_h$  predictions for most compounds were within 2-fold of observed *in vivo* values when protein binding was excluded, and estimated  $f_{m,AO}$  was within 25% of literature or clinical data for zaleplon

(TruVivo 0.58 vs. human 0.68) and zoniporide (TruVivo 0.78 vs. human 0.60). These results support using TruVivo as an early drug development tool for more accurate AO-mediated clearance and drug–drug interaction risk prediction.

## High and Sustained AO Activity

TruVivo sustained elevated aldehyde oxidase (AO) activity for 14 days—enabling accurate evaluation of slowly metabolized compounds. Compared to other models, TruVivo's AO activity was approximately 10X higher than HepatoPac cocultures and 2.6X higher than plated monocultures. Unlike suspended hepatocytes, which typically lose activity within hours, TruVivo maintained metabolic endurance far beyond the conventional window.



**Figure 1.** AO substrate metabolite formation is stable in TruVivo over 14 days. Higher activity measured in TruVivo compared to suspension cultures of the same donor. Data represent  $\pm$ SD of 3 replicates. Figure reproduced from portions of Figure 2 with permissions under an [open-access license](#)<sup>1</sup>.

## Improved Low Clearance Compound Predictions

TruVivo outperformed traditional hepatocyte models, delivering hepatic clearance predictions within 2-fold of *in vivo* values for four well-characterized AO substrates, carbazeran, zoniporide, zaleplon, and O6-benzylguanine.

## Relevant AO Expression and Reliable $CL_h$ Predictions

TruVivo demonstrated physiologically relevant AO activity, generating  $f_{mAO}$  values within 25% of published *in vivo* or accepted *in vitro* benchmarks using the AO-selective inhibitor hydralazine. This alignment suggests that AO activity in TruVivo more closely mirrors human physiological levels, enabling more accurate hepatic clearance ( $CL_h$ ) predictions.

Compound	$CL_h$ (mL/min/kg), Without $f_{u,p}$		$CL_h$ (mL/min/kg), With $f_{u,p}$		Observed <i>in vivo</i> $CL_h$ (mL/min/ kg)
	96-well		96-well		
	WSM	PTM	WSM	PTM	
Carbazeran	16.8	20.6	5.7	6.54	37.6
Zoniporide	13.5	17.5	8.25	10	17.1
Zaleplon	9.3	11.5	6.49	7.58	15.5
O6-BG	13.3	17.3	3.61	3.94	13.3
XK-469	0.659	0.669	0.00408	0.00408	0.039
Number of Predictions Within					
Twofold of <i>in vivo</i> value	3/5	4/5	0/5	2/5	
Threefold of <i>in vivo</i> value	4/5	4/5	2/5	2/5	

**Table 1.** Summary of TruVivo *in vitro*  $CL_{int}$  values to predicted *in vivo* plasma  $CL_h$  values using the WSM or the PTM, either incorporating or omitting  $f_{u,p}$  from the equation. Re-created from Table 1 with permission under an [open-access license](#)<sup>1</sup>.

## References

1. **Byer-Alcorace, A., Thomas, C., Taub, M. E., & Piekos, S. (2025).** Improved clearance predictions for aldehyde oxidase substrates using a novel triculture human hepatocyte model. *Drug Metabolism and Disposition*, 53(4), 100051–100051. <https://doi.org/10.1016/j.dmd.2025.100051>

