# The Effects of Organ Preservation Solution on Aldehyde Oxidase and Xanthine Oxidase Activity in Pooled Human Liver S9

Stephanie Helmstetter[1], Kevin C. Lyon[2], Phyllis Yerino[1], Aaron Hilgedick[1], Nicholas M. Hatfield[1], Bill Ewy[1], Zell Woodworth[1], Forrest Stanley[1], David B. Buckley[3], and Brian W. Ogilvie[1]
XenoTech, LLC [1], PRA Health Sciences[2], and Roivant Sciences, Inc.[3]

## INTRODUCTION

Non-transplant quality human livers donated for research are a common source of both cellular and subcellular material utilized in *in vitro* drug metabolism studies. At the time of organ recovery, livers are flushed with an ice-cold perfusion and storage solution that preserves the tissue during delivery from the recovery site to the research facility. The two most commonly used cold storage solutions are UW (University of Wisconsin) solution and Custodiol HTK (histidine tryptophan ketoglutarate) solution. One notable difference between the two solutions is the presence of 1 mM allopurinol in UW solution. It has been previously shown that 1 mM allopurinol inhibits xanthine oxidase (XO) activity, but not aldehyde oxidase (AO) in human liver cytosol [(1)]. Exposure of human livers to allopurinol during perfusion and cold storage could lead to underestimation of the contribution of xanthine oxidase in the metabolism of new chemical entities. In the present study, we evaluated xanthine oxidase and aldehyde oxidase activities in S9 fractions prepared from human livers that were flushed and stored in UW solution or HTK solution. To determine whether UW or HTK affected other drug metabolizing enzymes, we examined the metabolism of selective marker substrates for the major cytochrome P450 (CYP) enzymes.

## METHODS

Human livers, which were harvested with the intent of transplantation but subsequently rejected for this purpose due to lack of proper match or tissue condition, were obtained through partnerships with non-profit Organ Procurement Organizations. Livers were flushed and stored for up to 24 hours with either UW or HTK solution. Upon arrival of the organ at XenoTech on wet ice, livers were snap-frozen and stored at -80°C until processing to subcellular fractions at a later date.

Individual and pooled human liver S9 fractions (n = 1 or n = 20) fractions were prepared internally from 20 livers flushed and stored with UW solution and 20 livers flushed and stored with HTK solution. Phthalazine, *p*-vanillin, and zaleplon were purchased from Sigma-Aldrich (St. Louis, MO) and 6-nitroquinazolinone was purchased from Santa Cruz Biotechnology (Dallas, TX). All CYP marker substrates were purchased from Sigma-Aldrich with the exception of Amodiaquine and S-Mephenytoin, which were purchased from US Pharmacopeia (Rockville, MD) and Toronto Research Chemicals (Ontario, Canada), respectively.

Each S9 sample was characterized for enzymatic activity according to the substrate concentrations and incubation times listed in **Table 1**. For aldehyde and xanthine oxidase activities, each liver S9 sample (0.05 - 0.25 mg/mL) was incubated in triplicate at 37 ± 1°C in potassium phosphate buffer (50 mM, pH 7.4) and marker substrate. To measure CYP activity, liver S9 fractions (0.05 mg/mL) were incubated in triplicate at 37 ± 1°C in potassium phosphate buffer (50 mM, pH 7.4), containing $MgCl_2$ (3.0 mM), EDTA (1.0 mM), NADP (1.0 mM), glucose-6-phosphate (5.0 mM), glucose-6-phosphate dehydrogenase (1 Unit/mL) and marker substrate. Metabolite formation was determined by LC-MS/MS methods with deuterated metabolites as internal standards.

**Table 1**

| Enzyme | Substrate Concentration | Incubation Time (minutes) |
|---|---|---|
| AO | Zaleplon (20 µM) | 30 |
| AO | Phthalazine (20 µM) | 1 |
| AO | *p*-Vanillin (20 µM) | 10 |
| XO | 6-Nitroquinazolinone (1 µM) | 5 |
| CYP1A2 | Phenacetin (80 µM) | 10 |
| CYP2A6 | Coumarin (50 µM) | 10 |
| CYP2B6 | Bupropion (500 µM) | 10 |
| CYP2C19 | *S*-Mephenytoin (400 µM) | 10 |
| CYP2C8 | Amodiaquine (80 µM) | 10 |
| CYP2C9 | Diclofenac (100 µM) | 10 |
| CYP2D6 | Dextromethorphan (80 µM) | 10 |
| CYP2E1 | Chlorzoxazone (500 µM) | 10 |
| CYP3A4/5 | Testosterone (250 µM) | 10 |
| CYP3A4/5 | Midazolam (30 µM) | 10 |
| CYP4A11 | Lauric acid (100 µM) | 10 |

## RESULTS

**Figure 1** illustrates CYP activities in pooled human liver S9 fractions (n=20 for each pool) prepared from livers cold-preserved in UW and HTK solutions. Results show negligible difference in CYP activities between livers stored in UW and HTK solutions.

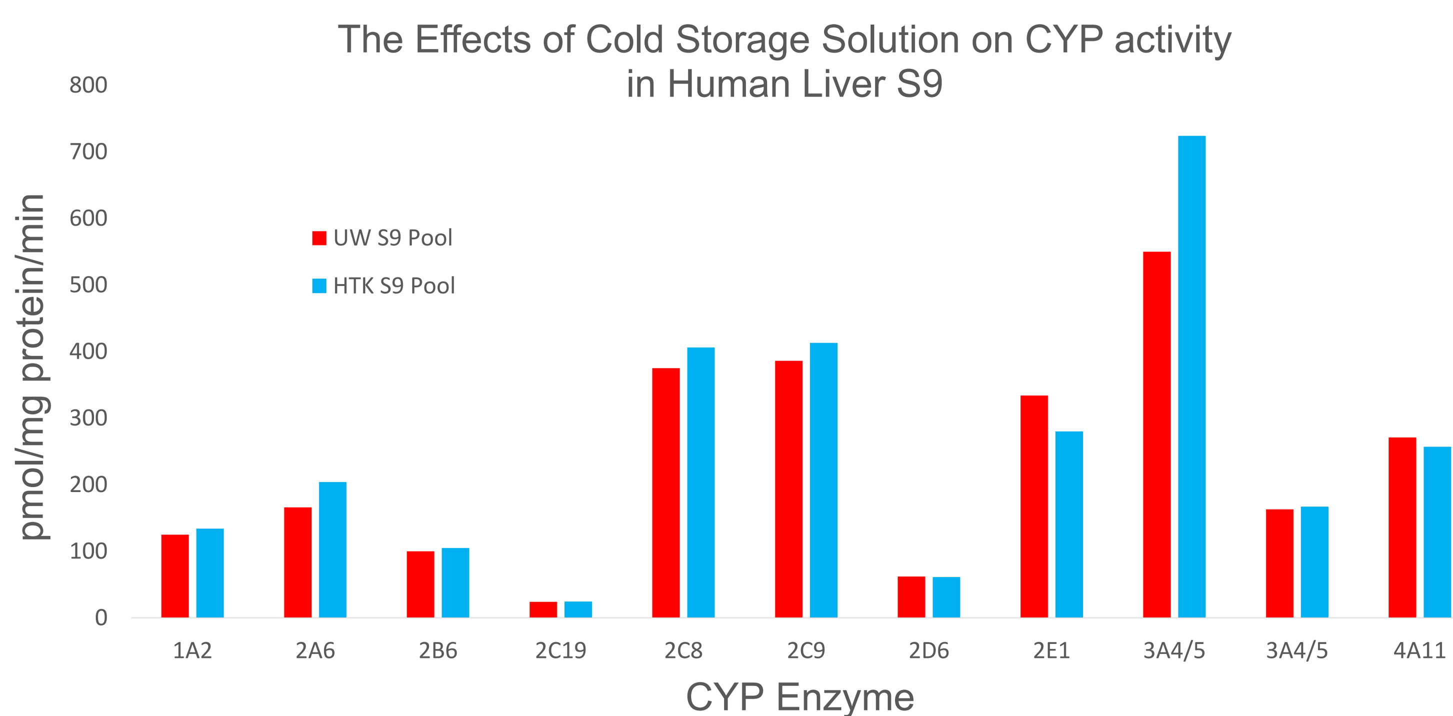
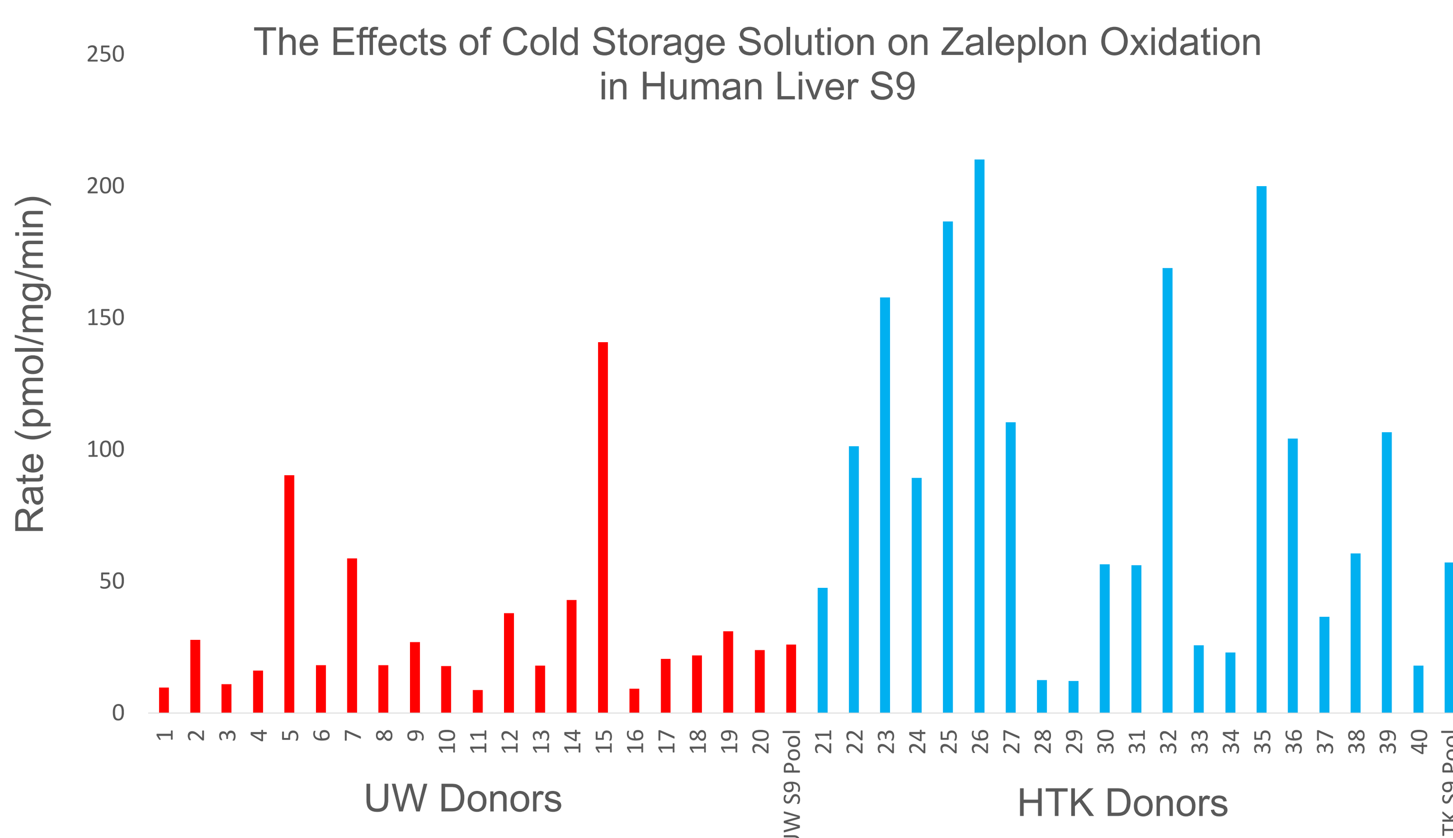



**Figure 2** illustrates zaleplon oxidation activity in human liver S9 fractions prepared from human livers stored in UW or HTK cold storage solution (20 individuals each, and n=20 for each pool). Across the various individual UW donors, the zaleplon oxidation activity ranged from 8.69 to 140 pmol/mg protein/min. The zaleplon oxidation activity of the pooled human liver S9 comprised of the twenty individual UW donors was 26.0 pmol/mg protein/min. In individual HTK donors, the zaleplon oxidation activity ranged from 12.1 to 210 pmol/mg protein/min. The pooled human liver S9 comprised of the twenty individual HTK donors exhibited an activity of 57.1 pmol/mg protein/min.




## RESULTS (continued…)

**Figure 3** illustrates phthalazine oxidation activity in human liver S9 fractions prepared from human livers stored in UW or HTK cold storage solution (20 individuals each, and n=20 for each pool). Across the various individual UW donors, the phthalazine oxidation activity ranged from 319 to 8640 pmol/mg protein/min. The phthalazine oxidation activity of the pooled human liver S9 comprised of the twenty individual UW donors was 1740 pmol/mg protein/min. In individual HTK donors, the phthalazine oxidation activity ranged from 330 to 12090 pmol/mg protein/min. The pooled human liver S9 comprised of the twenty individual HTK donors exhibited an activity of 3820 pmol/mg protein/min.

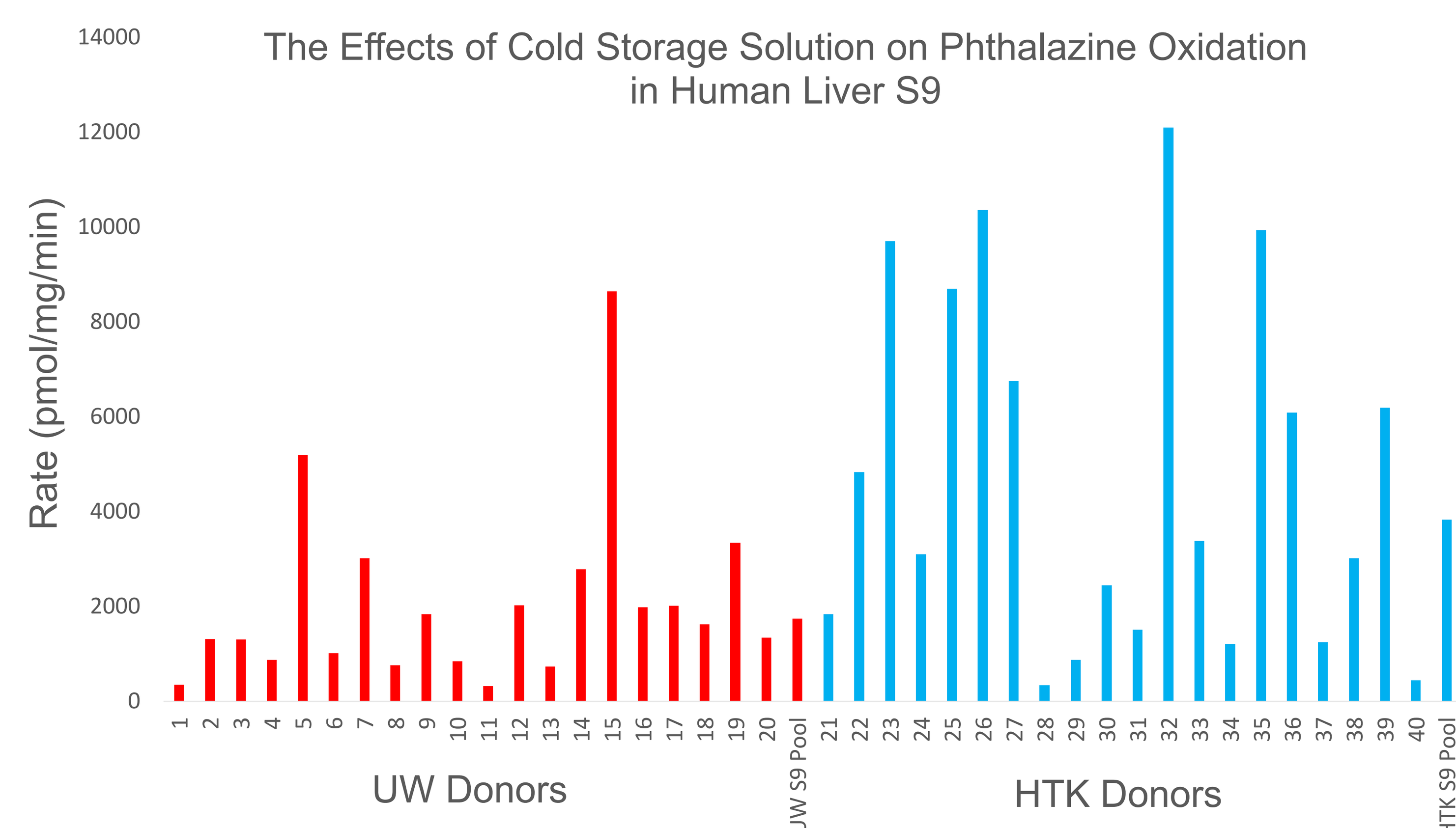



**Figure 4** illustrates *p*-vanillin oxidation activity in human liver S9 fractions prepared from human livers stored in UW or HTK cold storage solution (20 individuals each, and n=20 for each pool). Across the various individual UW donors, the *p*-vanillin oxidation activity ranged from 89.4 to 1230 pmol/mg protein/min. The p-vanillin oxidation activity of the pooled human liver S9 comprised of the twenty individual UW donors was 323 pmol/mg protein/min. In individual HTK donors, the *p*-vanillin oxidation activity ranged from 187 to 2860 pmol/mg protein/min. The pooled human liver S9 comprised of the twenty individual HTK donors exhibited an activity of 990 pmol/mg protein/min.

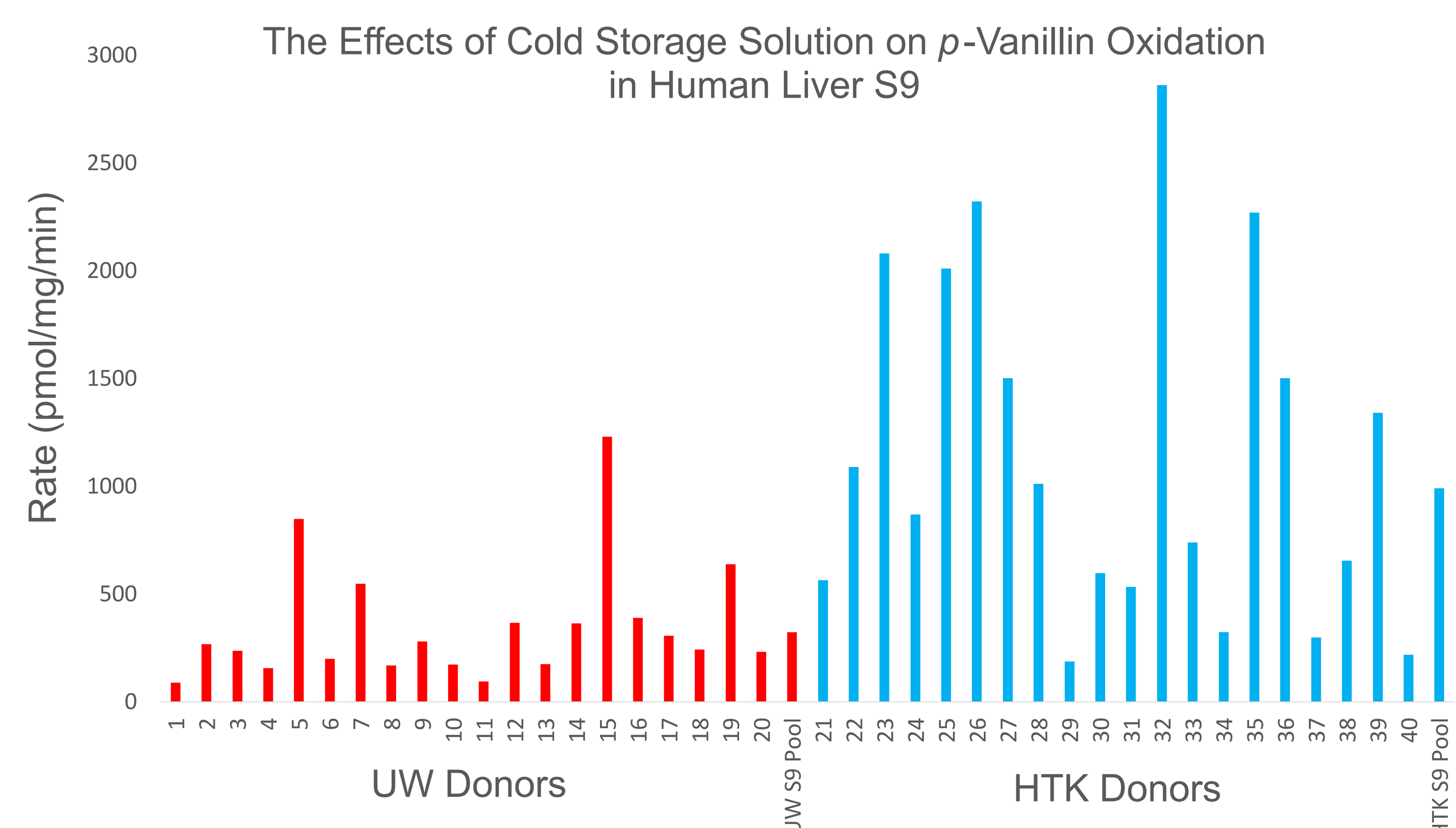
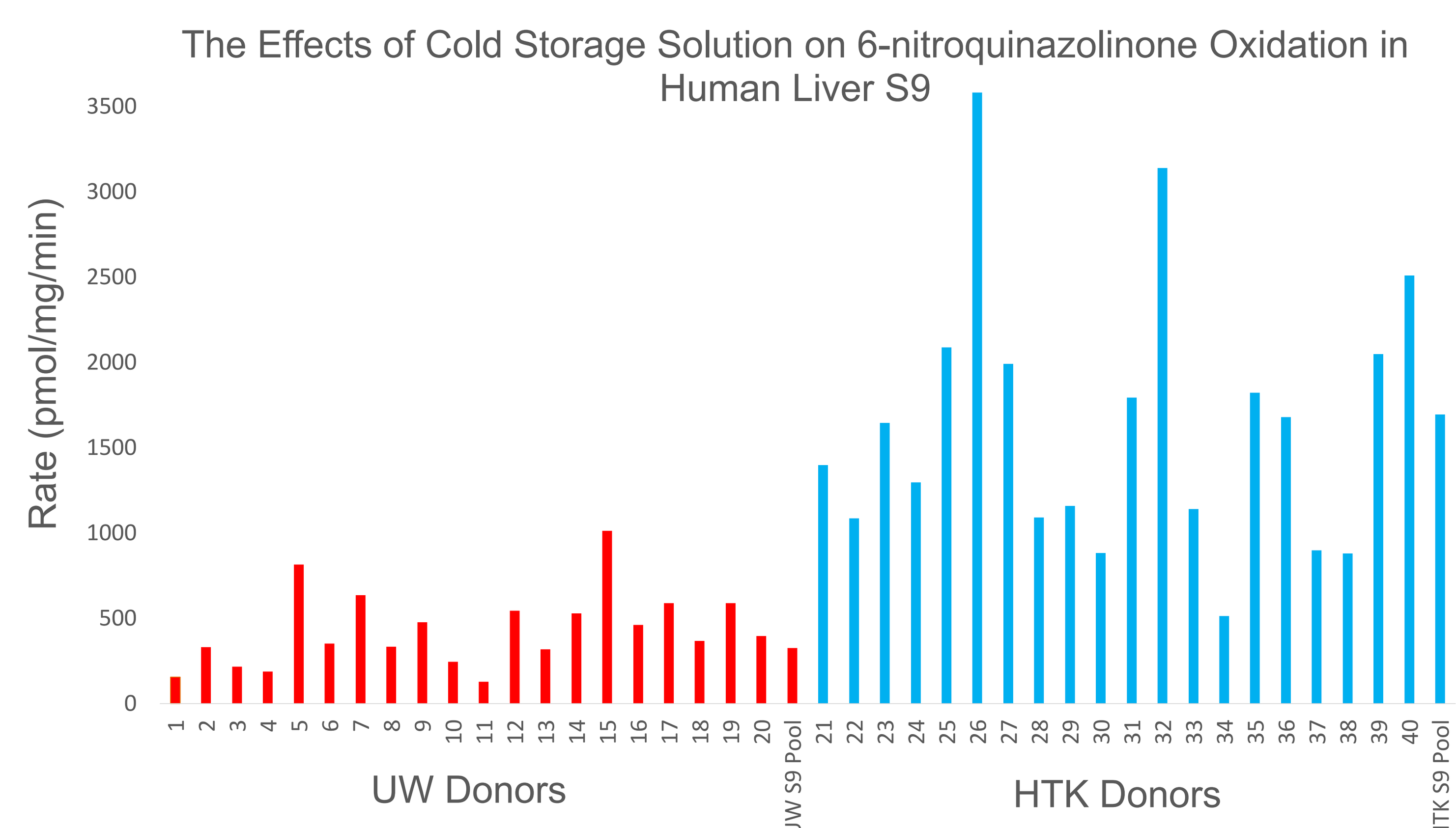



**Figure 5** illustrates 6-nitroquinazolinone oxidation activity in human liver S9 fractions prepared from human livers stored in UW or HTK cold storage solution (20 individuals each, and n=20 for each pool). Across the various individual UW donors, the 6-nitroquinazolinone oxidation activity ranged from 127 to 1012 pmol/mg protein/min. The 6-nitroquinazolinone oxidation activity of the pooled human liver S9 comprised of the twenty individual UW donors was 327 pmol/mg protein/min. In individual HTK donors, the 6-nitroquinazolinone oxidation activity ranged from 512 to 3580 pmol/mg protein/min. The pooled human liver S9 comprised of the twenty individual HTK donors exhibited an activity of 1696 pmol/mg protein/min.




## CONCLUSIONS

- Aldehyde oxidase activity, as measured by the oxidation of zaleplon to 5-oxo-zaleplon, phthalazine to 1-phthalazinone, and *p*-vanillin to vanillic acid was on average 2- to 3-fold higher in human liver S9 prepared from HTK-preserved livers than UW-preserved livers.
- Xanthine oxidase, as measured by the oxidation of 6-nitroquinazolinone to 6-nitroquinazolinedione, was on average over 5-fold higher in human liver S9 prepared from HTK-preserved livers than UW-preserved livers.
- Preservation of livers in HTK or UW cold storage solutions did not lead to a significant difference in CYP activity.
- These data indicate that a preparation of pooled human liver S9 prepared from tissue that is cold-preserved in HTK solution has increased xanthine oxidase and aldehyde oxidase activity with little effect on major CYP activity compared with S9 prepared from livers preserved in UW solution.

## REFERENCES

1. Barr JT, Choughule KV, Nepal S, Wong T, Chaudhry AS, Joswig-Jones CA, Zientek M, Strom SC, Schuetz EG, Thummel KE, and Jones JP (2014). Why do most human liver cytosol preparations lack xanthine oxidase activity? *Drug Metab Disp* (**42**):695-699.

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