NEW DATA AND ANALYSES ON CHLOROPRENE

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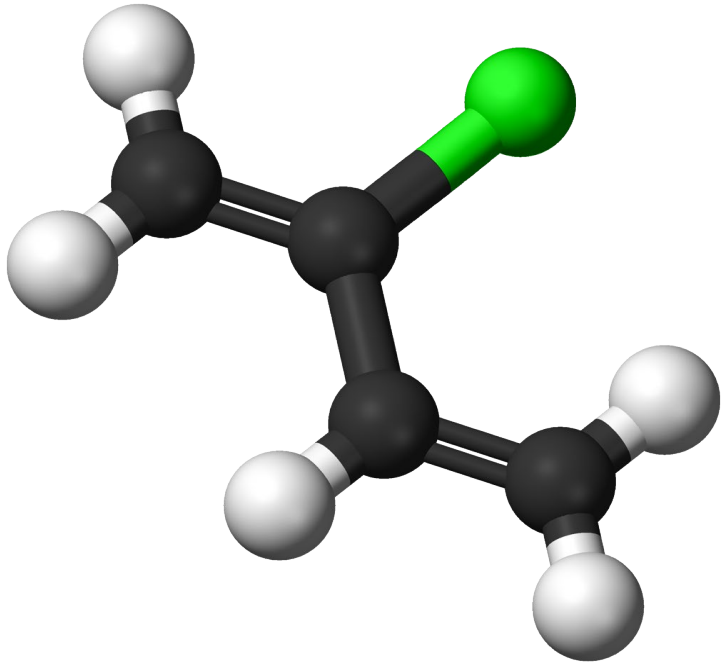


RAMBOLL

INTRODUCTION

- Denka Performance Elastomer LLC and Ramboll scientists have been working cooperatively with USEPA for several years to bring the best science forward to inform the PBPK model for chloroprene and its consideration in risk assessment.
- The presentation today is intended to:
  - Provide results from new epidemiological studies reporting recent evidence for cancer risk in workers and the general population
  - Provide results from recent analyses with the PBPK model to address the key uncertainties noted by the USEPA peer reviewers in their report (USEPA 2020)
  - Seek guidance from USEPA on next steps for PBPK model development/documentation
  - Seek guidance on how we move the process forward (RFC versus model documentation)
- We continue to appreciate USEPA's time and input into this process and look forward to your recommendations for next steps.

ANALYSIS OF NEW EPIDEMIOLOGICAL DATA

- The newest, best-quality occupational studies (Marsh et al. 2007 and 2021) show no increase in risk cancers for exposed employees
  - Occupational cohorts are much more highly exposed than area residents
  - Marsh et al. 2021 (update of 2007) found that chloroprene-exposed workers had lower rates of death due to respiratory and liver cancers and all cancers (combined) compared with residents of the areas around the plants studied
  - Strengthens the conclusions of decreased cancer risks from previous analyses

- Louisiana Tumor Registry, 2013-2017 shows lower risk of cancer for residents near the Denka facility vs. Louisiana
  - No increase in incidence of lung/bronchus, liver, or all cancers in Industrial Corridor parishes vs. Louisiana
  - St. John the Baptist parish incidence rates for lung/bronchus, liver, and all cancers (combined) are below the average for Louisiana

ANALYSIS OF NEW EPIDEMIOLOGICAL DATA

- Community assessment (Nagra et al. 2021) incorrectly estimated elevated 23-year period prevalence of cancer
  - Did not aim to produce objective science: ”The goals of the study were (1) to determine the overall health status of a large sample of residents living in the area of the Denka facility, (2) to assess the relationship between household proximity to the Denka facility and reported illness, and ***(3) to advance the advocacy objectives of Concerned Citizens by collecting and analyzing data that might be useful in the group’s efforts to compel Denka to adhere to the EPA’s 0.2 mg/m3 guideline for maximum chloroprene air concentration.***"
  - Incorrect epidemiological and statistical methods yield elevated 23-year period prevalence of all cancers (combined) in the census tract closest to the Denka plant (“zone 1”)
  - Application of correct, standard methods to the same data show no increase in prevalence of all cancers (combined) in the same census tract

RESULTS OF NEW PBPK MODEL ANALYSES

- Following the peer review of the chloroprene PBPK model, Ramboll has conducted additional investigations to assess the key uncertainties noted by the reviewers
  - Evaluation of the propagation of uncertainty in the estimated value of the mass transfer parameter, Kgl, to the metabolism parameter estimates and the resulting model predictions
  - Evaluation of the uncertainty in the estimated human lung:liver metabolism ratio, A1
  - Consideration of the potential impact of species differences in the downstream detoxification processes on tissue exposure to the epoxides of chloroprene

PEER REVIEW RECOMMENDATION: CONDUCT A SENSITIVITY ANALYSIS ON KGL

- Recommendation: Several reviewers felt there was too much uncertainty regarding the value of a parameter (Kgl) used to describe the mass-transfer resistance in the analysis of the previously published chloroprene *in vitro* studies (Himmelstein et al. 2004) that were used to estimate the metabolism parameters in the PBPK model. They recommended additional analyses should be conducted to determine the sensitivity of the model predictions to this parameter.
- Ramboll response: We conducted a sensitivity analysis of the impact of Kgl on estimates of the metabolism parameters, dose metrics and risk estimates, using the same MCMC approach provided in the Chloroprene PBPK Model Documentation (Ramboll 2020) for the USEPA peer review of the model.
  - Estimated metabolism parameters using Kgl values of 0.175 (the lowest value of Kgl for which the MCMC analysis was able to converge), 0.22, 0.44, 0.88, and 1000 (well mixed) determined the impact of the resulting metabolism parameters on the dose metrics and risk estimates.
  - Determined the goodness of fit of the *in vitro* model to the metabolism data using Kgl values of 0.11, 0.22, 0.44, 0.88 and 1000.

SENSITIVITY OF METABOLISM PARAMETERS TO KGL

	Female Mouse Liver		Female Mouse Lung		Human Liver		Human Lung (A1)	
Kgl (L/hr)	Vmaxc	Km	Vmaxc	Km	Vmaxc	Km	Vmax	Km
0.055	ND	ND	0.12	0.18	ND	ND	ND	ND
0.11	ND	ND	0.12	0.20	12.4	0.016	0.0026	0.016
0.175	7.68	0.032	0.12	0.21	13.0	0.024	0.0027	0.024
0.22	7.99	0.040	0.12	0.21	13.1	0.026	0.0028	0.026
0.44	8.43	0.054	0.12	0.21	13.5	0.032	0.0029	0.032
0.88	8.59	0.061	0.12	0.21	13.7	0.035	0.0029	0.035
1000	8.74	0.068	0.12	0.21	13.9	0.038	0.0029	0.038

ND: not determinable

Approximately 2-fold variation in estimates of Km in human liver/lung and mouse liver

SENSITIVITY OF GOODNESS OF FIT TO KGL

	Female Mouse Lung				Female Mouse Liver				Human Liver			
KGL	Vmax	Km	Relative* Error	Ratio to KGL = 0.22	Vmax	Km	Relative Error	Ratio to KGL = 0.22	Vmax	Km	Relative Error	Ratio to KGL = 0.22
0.175	0.022	2.369	1.18	1.017	0.101	0.365	8.39	1.012	0.049	0.269	1.25	1.582
0.22	0.022	2.369	1.16	**1.000**	0.105	0.448	8.11	**1.000**	0.050	0.299	0.79	**1.000**
0.44	0.021	2.368	1.39	1.198	0.111	0.615	8.00	0.993	0.051	0.361	1.17	1.481
0.88	0.021	2.339	1.27	1.095	0.113	0.691	7.92	0.992	0.052	0.395	0.75	0.949
1000	0.021	2.361	1.27	1.095	0.115	0.771	7.86	0.988	0.053	0.429	0.33	0.418

- Relative sum of squares: $\sum((\text{predicted}_i - \text{observed}_i)^2/(\text{observed}_i)^2)$

- **Kgl = 0.22 provides the best fit to the data in the mouse lung**
- **The fit to the mouse and human liver is slightly better at higher values of Kgl**
  - **Suggesting there may not have been a transport limitation in the experimental studies**

SENSITIVITY OF DOSE METRICS TO KGL

KGL		0.175		0.22		0.44		0.88		1000	
Dose Metric	**Concentration**	Amt. Metab. Liver	Amt. Metab. Lung	Amt. Metab. Liver	Amt. Metab. Lung	Amt. Metab. Liver	Amt. Metab. Lung	Amt. Metab. Liver	Amt. Metab. Lung	Amt. Metab. Liver	Amt. Metab. Lung
Female Mouse	**12.8 ppm**	1.16	0.88	1.16	0.85	1.16	0.85	1.16	0.86	1.15	0.86
	32 ppm	2.96	1.31	2.96	1.29	2.95	1.29	2.94	1.29	2.94	1.29
	80 ppm	7.68	1.71	7.66	1.69	7.64	1.69	7.62	1.69	7.61	1.69
Human	**1 ug/m3**	**3.56E-05**	**3.64E-06**	**3.56E-05**	**3.49E-06**	**3.57E-05**	**2.94E-06**	**3.57E-05**	**2.69E-06**	**3.57E-05**	**2.49E-06**

- **Mouse dose metrics unaffected**
- **Approximately 30% variation in estimates of dose metric for human lung**

SENSITIVITY OF RISK ESTIMATES TO KGL

BMDL: Female mouse lung dose metric - 2-stage (incidental unknown animal excluded)							
Risk Level	**KGL**	**LL**	**AIC**	**BMD (umole/gram lung tissue/ day)**	**BMDL (umole/gram lung tissue/day)**	**Internal continuous Human expsoure at 1 ug/m3**	**IUR Per ug/m3**
0.01	**0.175**	-82.900	173.80	0.0777	0.0097	3.64E-06	**3.7E-06**
0.01	**0.22**	-82.811	173.62	0.0757	0.0090	3.49E-06	**3.9E-06**
0.01	**0.44**	-82.811	173.62	0.0757	0.0090	2.94E-06	**3.3E-06**
0.01	**0.88**	-82.852	173.71	0.0762	0.0093	2.69E-06	**2.9E-06**
0.01	**1000**	-82.852	173.71	0.0762	0.0093	2.49E-06	**2.7E-06**

- **Kgl = 0.22 results in highest risk estimate**
- **Results support approach used in Clewell et al. (2020)**

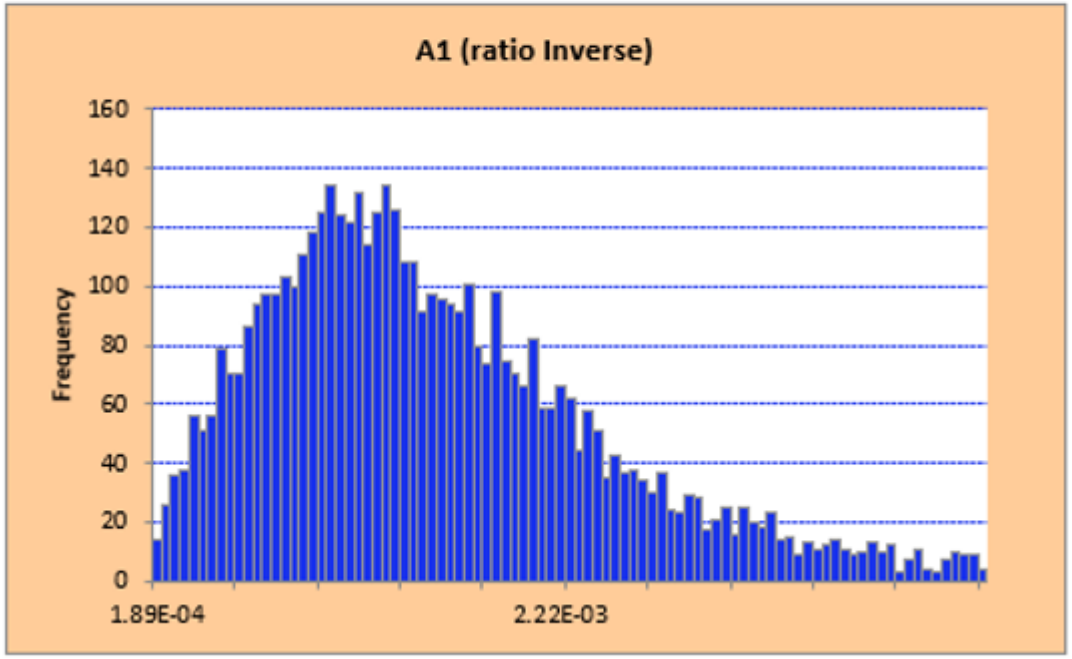
PEER REVIEW RECOMMENDATION: PERFORM AN UNCERTAINTY ANALYSIS ON A1

- Recommendation: Consider the range of values the parameter A1 can take and evaluate its impact as a part of supplemental uncertainty analysis
- Ramboll response: We conducted a multi-faceted analysis of the uncertainty in the estimate of A1 on risk estimates
  - Estimated the 95% confidence interval for A1 based on the data in Lorenz at al. (1984)
  - Performed a literature review to support estimation of an A1 for chloroprene based on CYP expression

95% CONFIDENCE INTERVAL FOR A1 DERIVED FROM LORENZ ET AL. 1984

Summary:
Entire range is from 1.66E-04 to 7.59E-03
Base case is 1.44E-03
After 5,000 trials, the std. error of the mean is 1.37E-05

Percentiles:		Forecast Values
2.5%		3.59E-04
25%		9.73E-04
50%		1.44E-03
95%		3.42E-03
98%		4.13E-03
Original Value:	1.43E-03	
Predicted mean:	1.64E-03	
Standard error of the mean:	1.37E-05	



- **Minimal uncertainty in estimate of A1**
- **Results support approach used in Clewell et al. (2020)**

ESTIMATION OF A1 FROM CYP ISOZYME EXPRESSION

Study	A1: 2E1+2F1	A1: 2E1+2F1+2A6
Bieche et al. 2007	0.005431199	0.005272981
Nishimura et al. 2003	0.00055948	0.001231242
Average:	**0.002995339**	**0.003252112**
Overall average A1:	**0.00312**	

* A1 from Lorenz et al. (1984): 0.00143 (0.00036 – 0.00413)

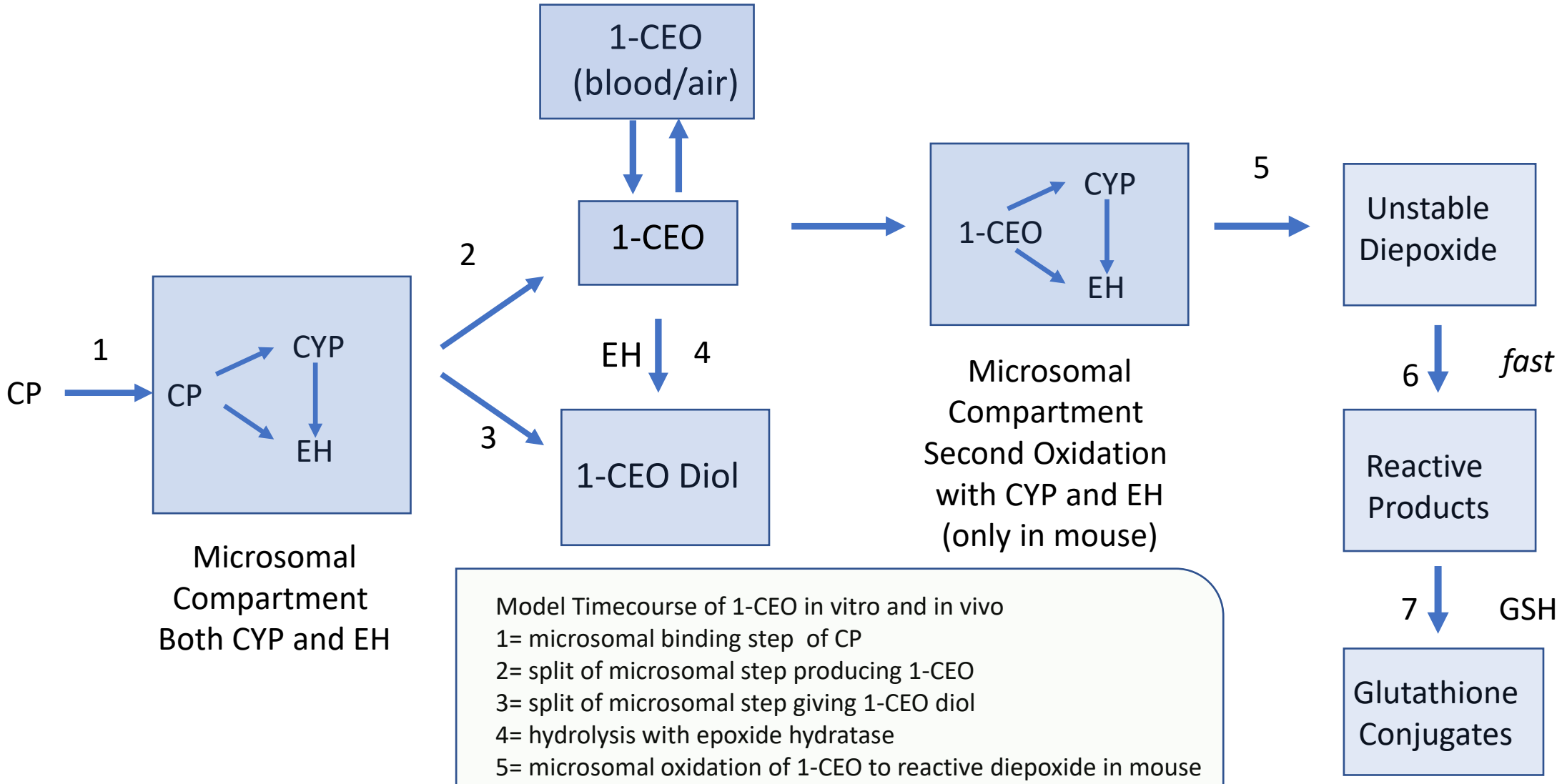
- **CYP isozyme expression data consistent with value of A1 (metabolic activity)**
- **Results support A1 approach used in Clewell et al. (2020)**

PEER REVIEW RECOMMENDATION: DETERMINE THE POTENTIAL IMPACT OF CHLOROPRENE METABOLITES ON HUMAN RISK

- Recommendation: Consider the potential impact of species differences in the downstream detoxication processes on tissue exposure to the epoxides of chloroprene
- Ramboll response: We have added a description of the formation and clearance of the chloroprene epoxides to the PBPK model and have used the available literature data to estimate the relevant parameters. The revised model will be exercised in a Monte Carlo simulation to estimate the potential impact of species differences in the clearance of the epoxides on estimates of human risk

Submodels for Stable Epoxides and Reactive Products formed by Chloroprene (CP) Oxidation

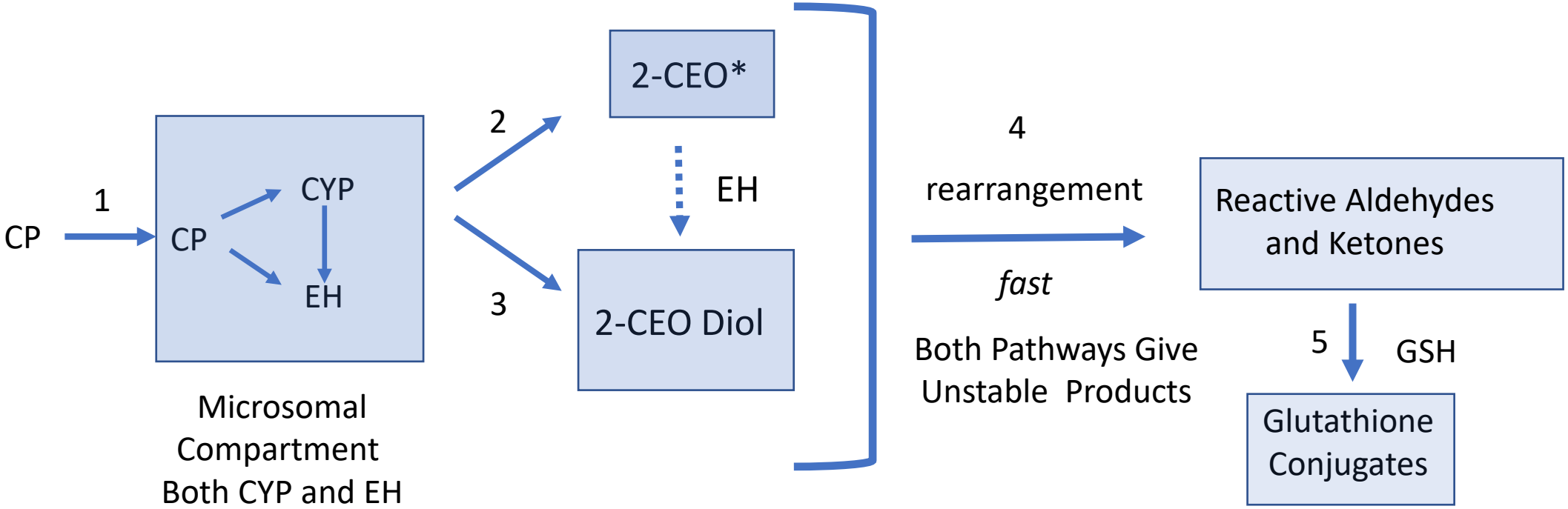
1-Chloroethylene Oxide is stable with an appreciable half-life in tissues. The pathway included in the submodel are:



Model Timecourse of 1-CEO in vitro and in vivo
1= microsomal binding step of CP
2= split of microsomal step producing 1-CEO
3= split of microsomal step giving 1-CEO diol
4= hydrolysis with epoxide hydratase
5= microsomal oxidation of 1-CEO to reactive diepoxide in mouse
6= rapid rearrangement steps
7=reaction of reactive products with GS

Submodels for Stable Epoxides and Reactive Products formed by Chloroprene (CP) Oxidation

2-Chloroethylene Oxide is unstable and short-lived. Fewer steps are needed in a 2-CEO submodel. Need to know the flux to reactive products but not time course of 2-CEO itself.



Model Time course of Flux in 2-CEO pathway in vitro and in vivo
1= microsomal binding step of CP
2= split of microsomal step producing 2-CEO
3= split of microsomal step giving 2-CEO
4 = rapid rearrangement of 2-CEO to reactive products
5 = Conjugation of reactive products with glutathione (GSH)

MODELING REACTIVE PRODUCTS

- We have parameterized the chloroprene epoxides/reactive products submodel using data from Himmelstein et al. (2004) and by estimating an upper bound on the half-life of 2-CEO, which is too reactive to measure *in vitro*.
- The GSH pathway does not contribute to 1-CEO clearance (Munter et al., 2003) and did not need to be included in the submodel.
- Only the 1-CEO is amenable to modeling for deriving a meaningful measure of concentration and area under the curve.
- The greatest impact of metabolism through the 2-CEO pathway is the production of large amounts of reactive products that consume GSH.
- The chloroprene epoxide/reactive products submodel is now being incorporated into Ramboll's chloroprene PBPK model. It is anticipated that 30-60 days will be needed to complete this extension of the PBPK model.

SUMMARY OF NEW DATA/ANALYSES

- ***Epidemiological Studies:***
  - Updates to both occupational studies and the Louisiana Tumor Registry continue to demonstrate no increase in cancer deaths in workers and no increase in incidence or prevalence of cancers in residents of St. John the Baptist Parish.
- ***New Analysis of the Chloroprene PBPK Model in Response to Peer Review Comments:***
  - ***Sensitivity Analysis of Kgl*** – the value for Kgl (0.22 L/hr) used in the Clewell et al. (2020) PBPK model for Chloroprene is the most consistent with the available data in both the mouse and the human and provides the most conservative estimate of risk.
  - ***Uncertainty Analysis of A1*** – statistical evaluation of the data used to characterize A1 in the Clewell et al. (2020) PBPK model for Chloroprene indicates minimal uncertainty in the estimate. Literature searches identified additional studies of CYP isozyme expression consistent with the current value used in Clewell et al. (2020) and providing additional support for the approach.
  - ***Modeling Chloroprene Epoxides*** – The chloroprene PBPK model has been extended to include a preliminary description of the reactive products generated from chloroprene. This extended model will be used to evaluate the potential impact of species differences in the clearance of the epoxides on estimates of human risk.

NEXT STEPS

- ***New Analysis of the Chloroprene PBPK Model in Response to Peer Review Comments:***
  - ***Modeling Chloroprene Epoxides*** – It is anticipated that 30-60 days will be needed to complete the extension of the PBPK model and the evaluation of the potential impact of species differences in the clearance of the epoxides on estimates of human risk.

- ***Moving Forward***
  - ***How should the revised PBPK model documentation be provided?***
  - ***Does the RFC remain the approach that should be used?***
    - ***Denka's preference would be to continue to work cooperatively with USEPA***
  - ***What are USEPA recommendations for next steps?***

- **We would like to again extend our appreciation for USEPA's time and guidance in applying the best science possible to the evaluation of chloroprene.**

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