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Supplemental Material

Filaggrin Polymorphisms and the Uptake of Chemicals through the Skin – A Human Experimental Study

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Table S1. Filaggrin null mutations with rs-id, and primer and probe pairs.

SNP	Probe functional allele	Probe null allele	Primer pairs
R501X (rs61816761)	CAGGCACGAGACAG	CAGGCATGAGACAG	Forward: 5′AGCACTGGAGGAAGACAAGGATC3′ Reverse: 5′ACCCTCTTGGGACGCTGAAT3′
2282del4 (rs41370446)	CACAGTCAGTGTCAG	CACAGTGTCAG	Forward 1: 5′TCCCGCCACCAGCTCC3′ Forward 2: 5′CCACTGACAGTGAGGGACATTCA3′ Reverse: 5′GGTGGCTCTGCTGATGGTGA3′
R2447X (rs138726443)	CACGAGACAGCTC	CATGAGACAGCTC	Forward: 5′CACGTGGCCGGTCAGCA3′ Reverse: 5′TCCTGACCCTCTTGGGACGT3′
S3247X (rs150597413)	CAGTCAAGGCACG	CAGTAAAGGCACG	Forward: 5′CCAGAAACCATCGTGGATCTG3′ Reverse: 5′TGCCTGATTGTCTGGAGCG3′

Table S2. The mode of ionization and transitions of the analyzed compounds and corresponding internal standards.

Chemical	Ionization mode	Quantifier ions	Qualifier ions	Auxiliary gas temperature	Ion spray voltage	Collison energy
OH-Pyrimethanil	+	216.1/107.0	216.1/159.2	650	5500	36
$^{2}H_{4}$-OH-Pyrimethanil	+	220.1/111.0	220.1/163.2	650	5500	36
Oxybenzone	+	229.1/150.9	229.1/105.0	600	4500	26
$^{2}H_{3}$-Oxybenzone	+	232.1/154.0	232.1/105.0	600	4500	26
1-hydroxy-pyrene	-	217.0/189.0	---	500	-4500	-48
$^{2}H_{9}$-1-hydroxy-pyrene	-	226.0/198.0	---	500	-4500	-48

Table S3. Between-run and between-batch precision of the analytical methods determined at different concentrations.

Precision	*n*	Concentrations (µg/L)	Mean (µg/L)	CV (%)	LOD (µg/L)
OH-pyrimethanil					0.1
Between-run	28	10	14	12	
	28	20	23	6.1	
Between-batch	79	range LOD to 10	5.0	6.3	
	446	range 10 to 200	62	5.9	
	102	range 200 to 2000	547	3.6	
Oxybenzone					0.2
Between-run	32	10	7.5	12	
	32	20	15	13	
Between-batch	119	range LOD to 10	5.5	6.3	
	444	range 10 to 200	60	6.8	
	89	range 200 to 1860	579	7.9	
1-OH-pyrene					0.2
Between-run	28	5	3.9	8.3	
	28	10	7.6	9.8	
Between-batch	484	range LOD to 5	1.3	5.0	
	79	range 5 to 30	11	4.1	

Table S4. Area under the urine excretion rate curve ($AUC_{(0–40h)}$), lag time for dermal absorption and dermal absorption rate constant by *FLG* genotype.

Chemical	Genotype	$AUC_{(0–40h)}$ (nmol, geometric mean, 95% c.i.)	*P*-value (*t*-test)	Lag time for dermal absorption (h, mean ± SD)	*P*-value (ANOVA)	Dermal absorption rate constant (h^{-1}, mean ± SD)	*P*-value (ANOVA)
Pyrimethanil	*FLG* null	1676; 1244, 2259	0.171[a]	0.20 ± 0.11	0.003	0.20 ± 0.05	0.028
	FLG wt	1279; 995, 1644		0.53 ± 0.32		0.17 ± 0.07	
Pyrene	*FLG* null	28.4; 22.8, 35.3	0.430[b]	0.88 ± 0.13	0.009	0.17 ± 0.06	0.24
	FLG wt	25.3; 21.0, 30.5		1.20 ± 0.25		0.15 ± 0.06	
Oxybenzone	*FLG* null	1160; 889, 1517	0.380[c]	0.10 ± 0.02	0.055	0.29 ± 0.08	0.004
	FLG wt	994; 790, 1250		0.13 ± 0.07		0.22 ± 0.09	

Note: *P*-values are for comparison between *FLG* null (n=22) and wt (n[oxybenzone]=30, n[pyrimethanil and pyrene]=31) using t-test for $AUC_{(0–40h)}$ in IBM SPSS and for lag time and dermal absorption rate constant using ANOVA, by default, in Monolix.

[a]R^2=0.036

[b]R^2=0.012

[c]R^2=0.015

Table S5. Area under the urine excretion rate curve ($AUC_{(0–40h)}$) adjusted for BMI and age, by *FLG* genotype.

Chemical	Genotype	$AUC_{(0–40h)}$ (nmol, geometric mean, 95% c.i.)	*P*-value (ANOVA)
Pyrimethanil	*FLG* null	1671; 1258, 2213	0.155[a]
	FLG wt	1279; 1004, 1629	
Pyrene	*FLG* null	28.6; 23.4, 35.1	0.370[b]
	FLG wt	25.4; 21.3, 30.2	
Oxybenzone	*FLG* null	1153; 899, 1479	0.381[c]
	FLG wt	997; 803, 1238	

Note: *P*-values are for comparison between *FLG* null (*n*=22) and wt (*n*[oxybenzone]=30, *n*[pyrimethanil and pyrene]=31).

[a] R^2=0.194

[b] R^2=0.209

[c] R^2=0.196

Table S6. Area under the urine excretion rate curve ($AUC_{(0–40h)}$), adjusted BMI and age, by *FLG* null and CNV genotype.

Chemical	Genotype[a]	$AUC_{(0–40h)}$ (nmol, geometric mean, 95% c.i.)	P-value (ANOVA)
Pyrimethanil	*FLG* null	1682; 1285, 2202	0.095[b,c]
	FLG wt CNV20–22	1318; 990, 1753	
	FLG wt CNV23–24	954; 602, 1510	
Pyrene	*FLG* null	28.6; 23.6, 34.6	0.331[d]
	FLG wt CNV20–22	26.1; 21.2, 31.9	
	FLG wt CNV23–24	21.6; 15.5, 29.9	
Oxybenzone	*FLG* null	1158; 912, 1475	0.348[e]
	FLG wt CNV20–22	995; 765, 1294	
	FLG wt CNV23–24	831; 552, 1256	

Note: *P*-values for comparisons are between *FLG* null (*n*=22), wt CNV20–22 (*n*[oxybenzone)=19, *n*[pyrimethanil and pyrene]=20) and wt CNV23–24 (*n*=8).

[a]Three participants do not have information about CNV.

[b]Significant pairwise comparison between *FLG* null and wt CNV23–24: *P*=0.037.

[c]R^2=0.291

[d]R^2=0.275

[e]R^2=0.260

Table S7. Lag time for dermal absorption and dermal absorption rate constant by *FLG* null and CNV genotype. Analysis adjusted for BMI, age and sex.

Chemical	Genotype[a]	Lag time (h, mean ± SD)	*P*-value (ANOVA)	Absorption rate constant (h^{-1}, mean ± SD)	*P*-value (ANOVA)
Pyrimethanil	*FLG* null	0.45 ± 0.04	0.00069	0.14 ± 0.03	0.017
	FLG wt CNV20–22	0.55 ± 0.10		0.14 ± 0.04	
	FLG wt CNV23–24	0.75 ± 0.19		0.11 ± 0.03	
Pyrene	*FLG* null	0.89 ± 0.15	0.0036	0.23 ± 0.09	0.026
	FLG wt CNV20–22	1.06 ± 0.17		0.23 ± 0.10	
	FLG wt CNV23–24	1.34 ± 0.32		0.14 ± 0.05	
Oxybenzone	*FLG* null	0.14 ± 0.18	0.012	0.22 ± 0.06	0.00069
	FLG wt CNV20–22	0.04 ± 0.04		0.20 ± 0.06	
	FLG wt CNV23–24	0.13 ± 0.17		0.13 ± 0.04	

Note: *P*-values for comparisons are between *FLG* null (*n*=22), wt CNV20–22 (*n*[oxybenzone]=19, *n*[pyrimethanil and pyrene]=20) and wt CNV23–24 (*n*=8).

[a]Three participants do not have information about CNV.

Table S8. Volume of the central compartment, rate constant from the central compartment to the peripheral compartment, rate constant from the peripheral compartment to the central compartment, and excretion rate constant by *FLG* genotype.

Chemical	Genotype	Volume of central compartment (L, mean ± SD)	*P*-value (ANOVA)	Rate constant K_{21}[a] (h^{-1}, mean ± SD)	*P*-value (ANOVA)	Rate constant K_{12}[b] (h^{-1}, mean ± SD)	*P*-value (ANOVA)	Excretion rate constant K (h^{-1}, mean ± SD)	*P*-value (ANOVA)
Pyrimethanil	*FLG* null	69.6±38.8	0.10	0.006±0.002	0.78	0.29±0.06	0.057	0.0001±0.00006	0.21
	FLG wt	93.6±59.5		0.006±0.002		0.33±0.07		0.0001± 0.0001	
Pyrene	*FLG* null	942±235	0.26	0.02±0.01	0.38	0.13±0.03	0.39	0.13±0.04	0.13
	FLG wt	1020±245		0.02±0.0008		0.13±0.02		0.16±0.06	
Oxybenzone	*FLG* null	79.5±34.0	0.18	0.03±0.01	0.59	0.17±0.04	0.99	0.18±0.04	0.31
	FLG wt	94.6±42.3		0.03±0.01		0.17±0.05		0.20±0.06	

Note: *P*-values are for comparison between *FLG* null (*n*=22) and wt (*n*[oxybenzone]=30, *n*[pyrimethanil and pyrene]=31).

[a]From the central to the peripheral compartment.

[b]From the peripheral to the central compartment.

Table S9. Volume of the central compartment, rate constant from the central compartment to the peripheral compartment, rate constant from the peripheral compartment to the central compartment, and excretion rate constant by *FLG* CNV genotype.

Chemical	Genotype[a]	Volume of central compartment (L, mean ± SD)	*P*-value (ANOVA)	Rate constant K_{21}[b] (h^{-1}, mean ± SD)	*P*-value (ANOVA)	Rate constant K_{12}[c] (h^{-1}, mean ± SD)	*P*-value (ANOVA)	Excretion rate constant K (h^{-1}, mean ± SD)	*P*-value (ANOVA)
Pyrimethanil	*FLG* null	80.5±56.2	0.026	0.007±0.003	0.86	0.26±0.04	0.23	0.006±0.01	0.62
	FLG wt CNV20–22	99.2±51.9		0.007±0.002		0.26±0.04		0.004±0.004	
	FLG wt CNV23–24	156±112		0.007±0.003		0.29±0.03		0.004±0.003	
Pyrene	*FLG* null	876±344	0.12	0.02±0.0008	0.61	0.12±0.04	0.77	0.17±0.02	0.48
	FLG wt CNV20–22	954±410		0.02±0.0006		0.12±0.03		0.18±0.04	
	FLG wt CNV23–24	1232±541		0.02±0.0005		0.11±0.02		0.18±0.02	
Oxybenzone	*FLG* null	71.5±32.4	0.12	0.01±0.006	0.40	0.31±0.02	0.78	0.05±0.02	0.20
	FLG wt CNV20–22	81.5±42.7		0.01±0.008		0.31±0.02		0.05±0.01	
	FLG wt CNV23–24	103±52.36		0.007±0.003		0.32±0.02		0.06±0.04	

Note: *P*-values for comparisons are between *FLG* null (*n*=22), wt CNV20–22 (*n*[oxybenzone)=19, *n*[pyrimethanil and pyrene]=20) and wt CNV23–24 (*n*=8).

[a]Three participants lack information about *FLG* CNV.

[b]From the central to the peripheral compartment.

[c]From the peripheral to the central compartment.

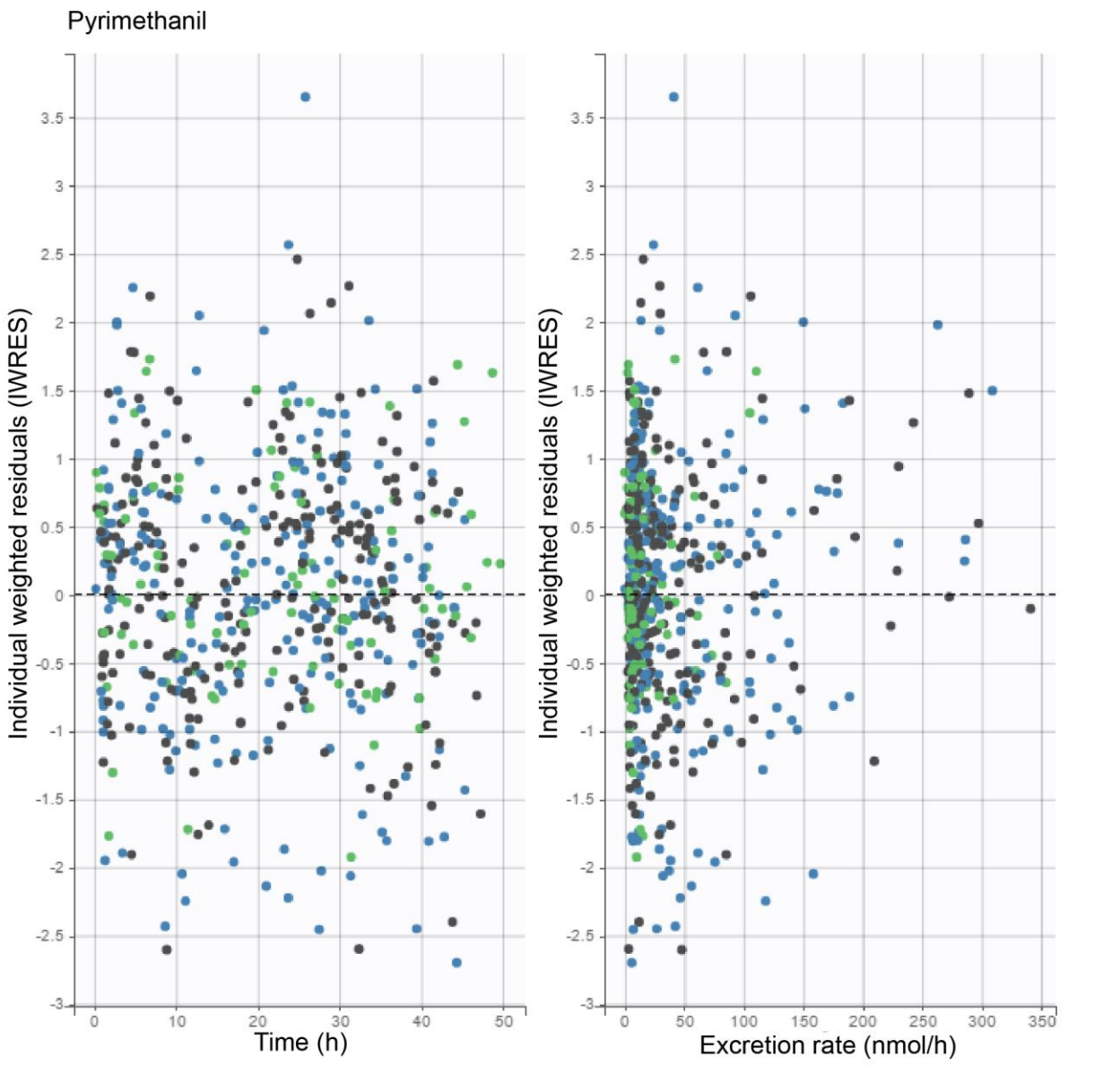


Figure S1. Residual plots for time point and excretion rate for pyrimethanil. Blue = *FLG* null, black = wt CNV20–22, green = wt CNV23–24.

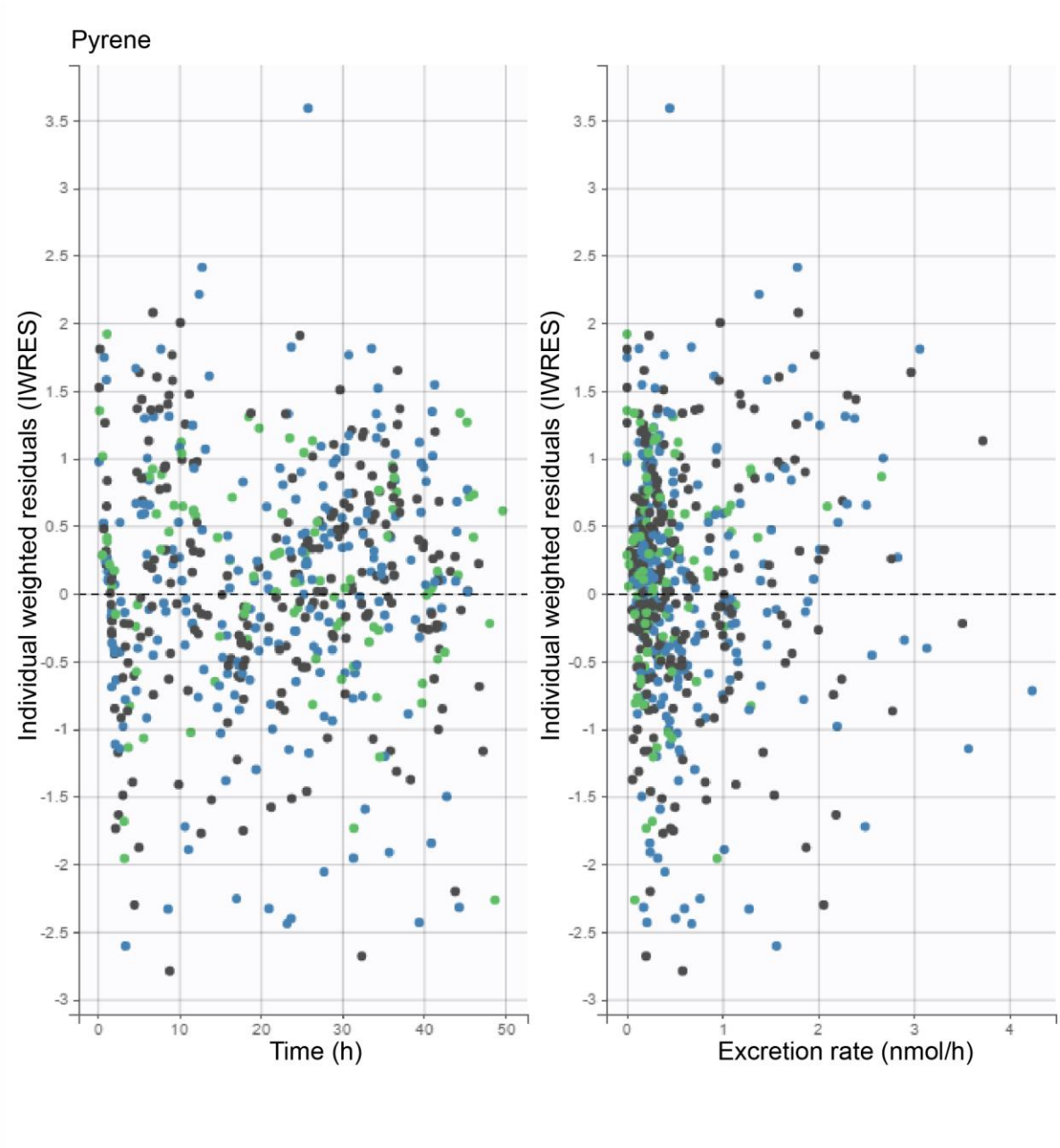


Figure S2. Residual plots for time point and excretion rate for pyrene. Blue = *FLG* null, black = wt CNV20–22, green = wt CNV23–24.

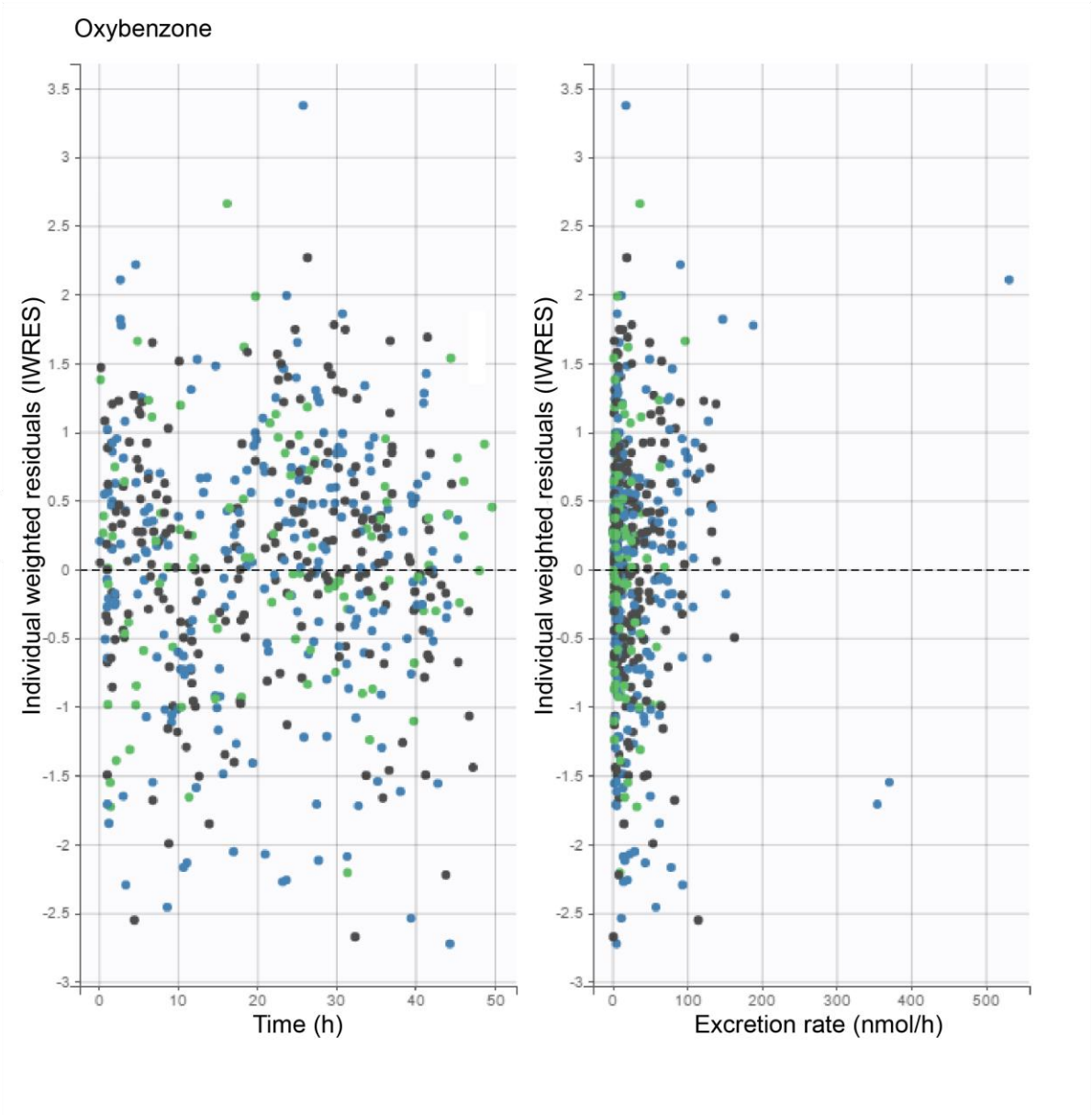


Figure S3. Residual plots for time point and excretion rate for oxybenzone. Blue = *FLG* null, black = wt CNV 20–22, green = wt CNV 23–24.

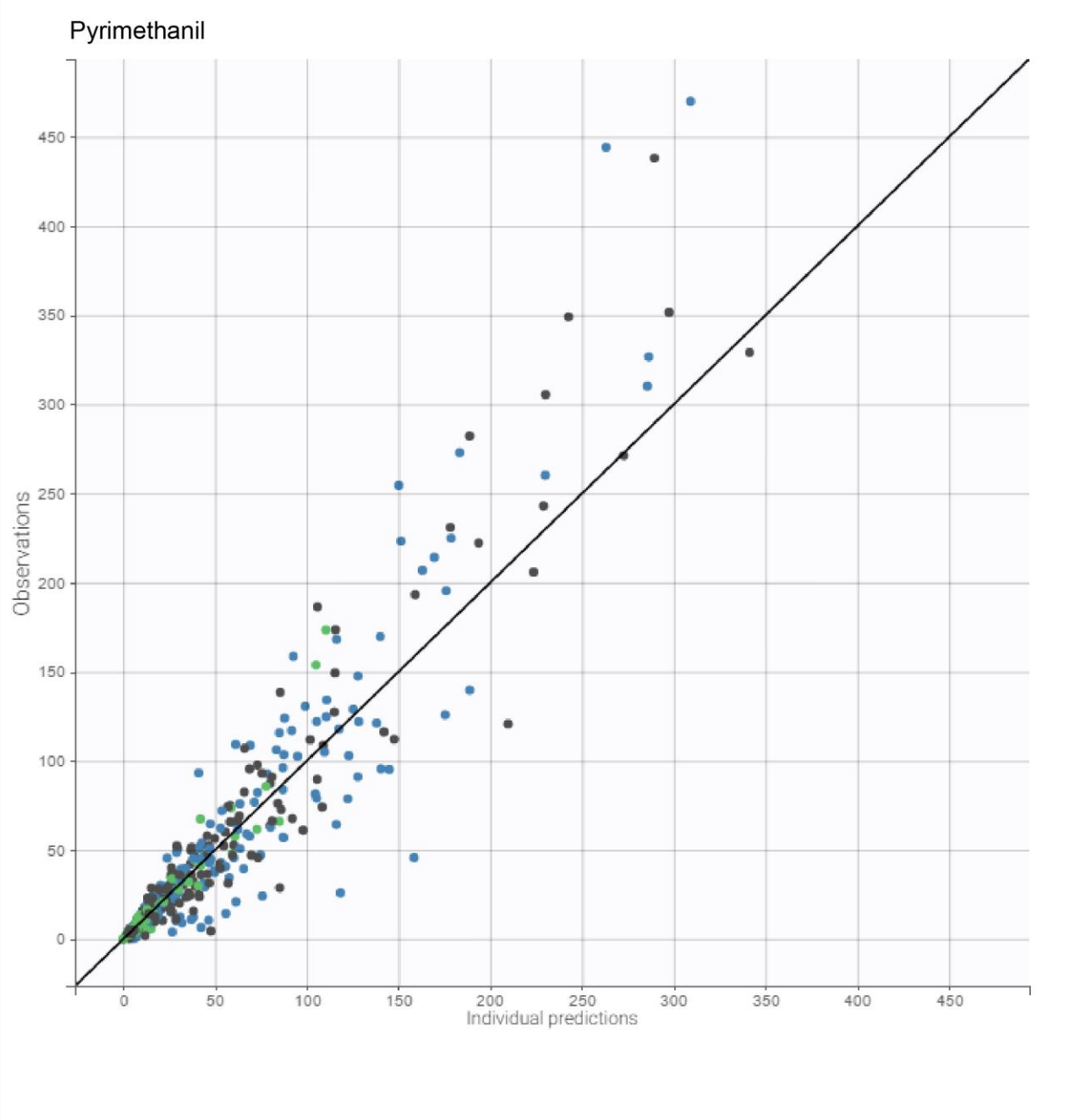


Figure S4. The predicted vs observed values for excretion rates for pyrimethanil. Blue = *FLG* null, black = wt CNV 20–22, green = wt CNV 23–24.

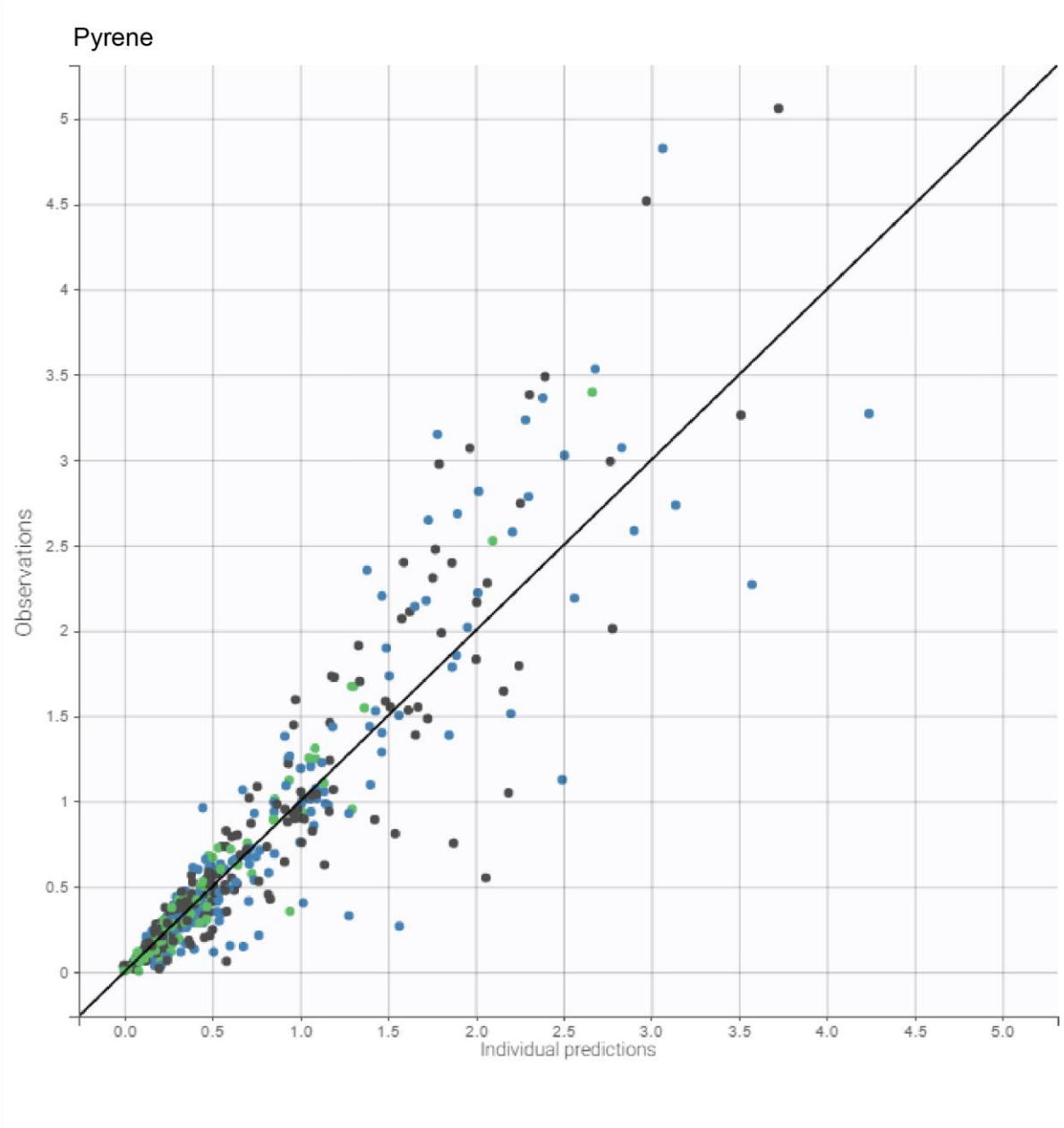


Figure S5. The predicted vs observed values for excretion rates for pyrene. Blue = *FLG* null, black = wt CNV 20–22, green = wt CNV 23–24.

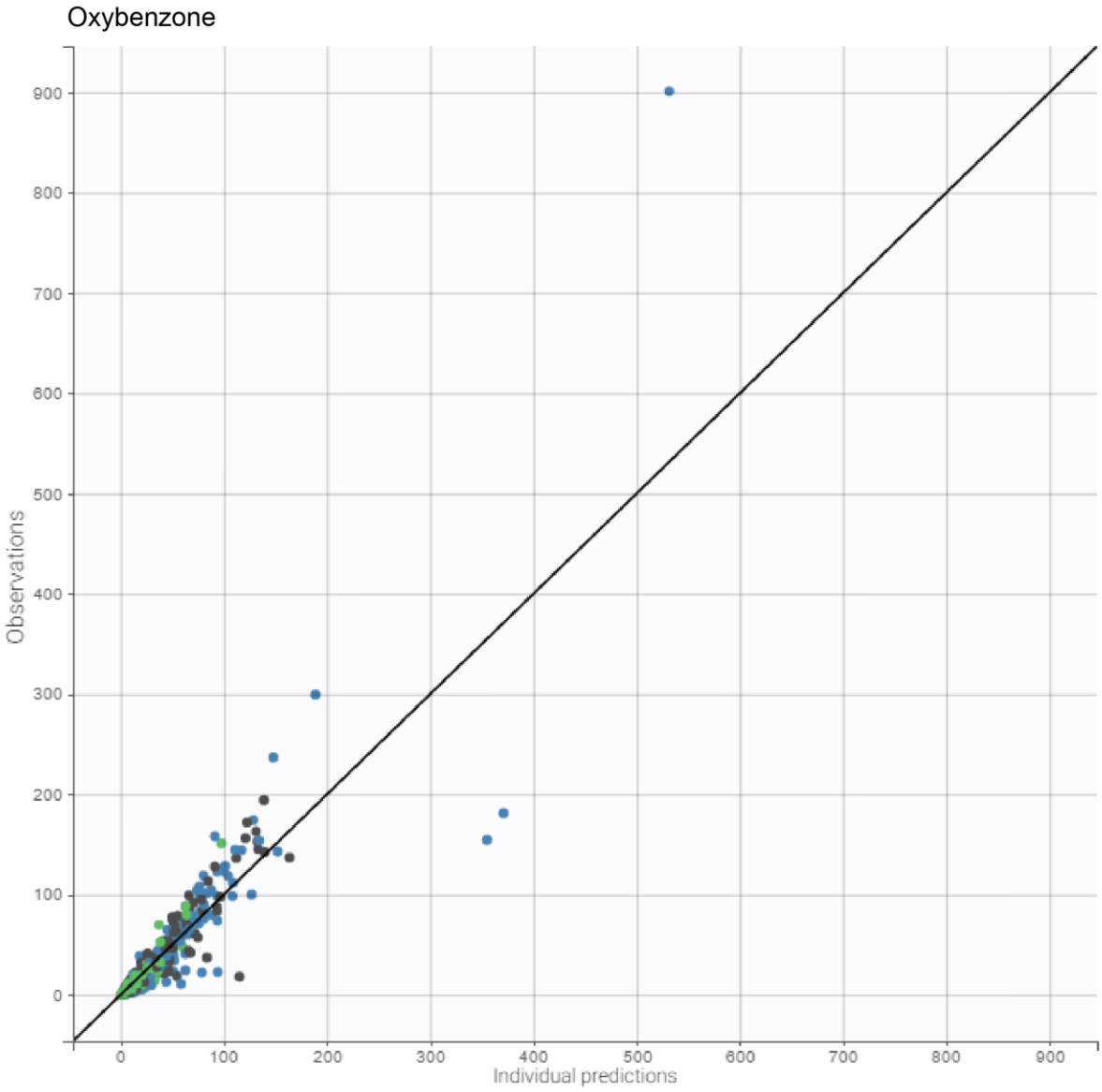


Figure S6. The predicted *vs* observed values for excretion rates for oxybenzone. Blue = *FLG* null, black = wt CNV 20–22, green = wt CNV 23–24.

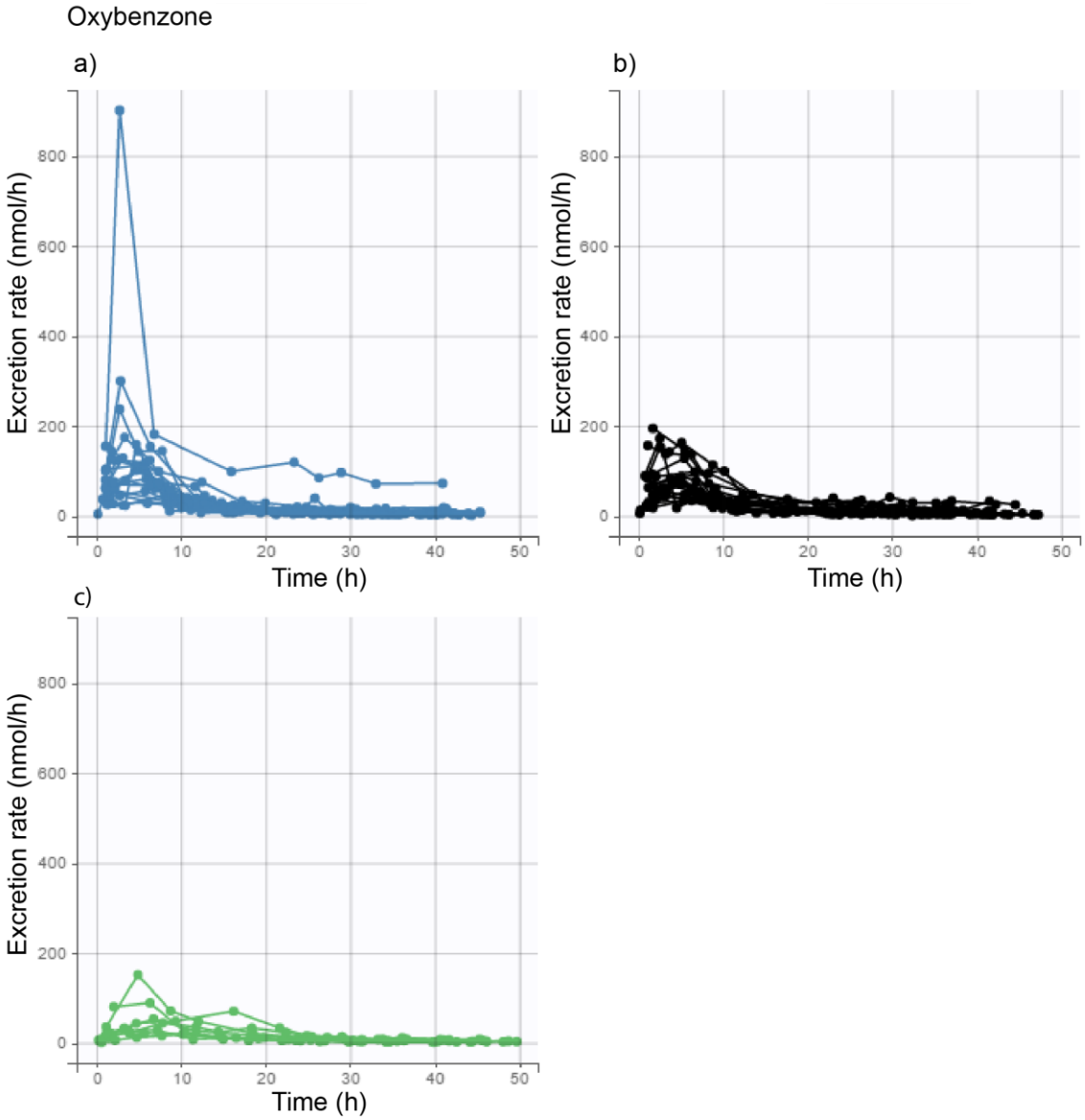
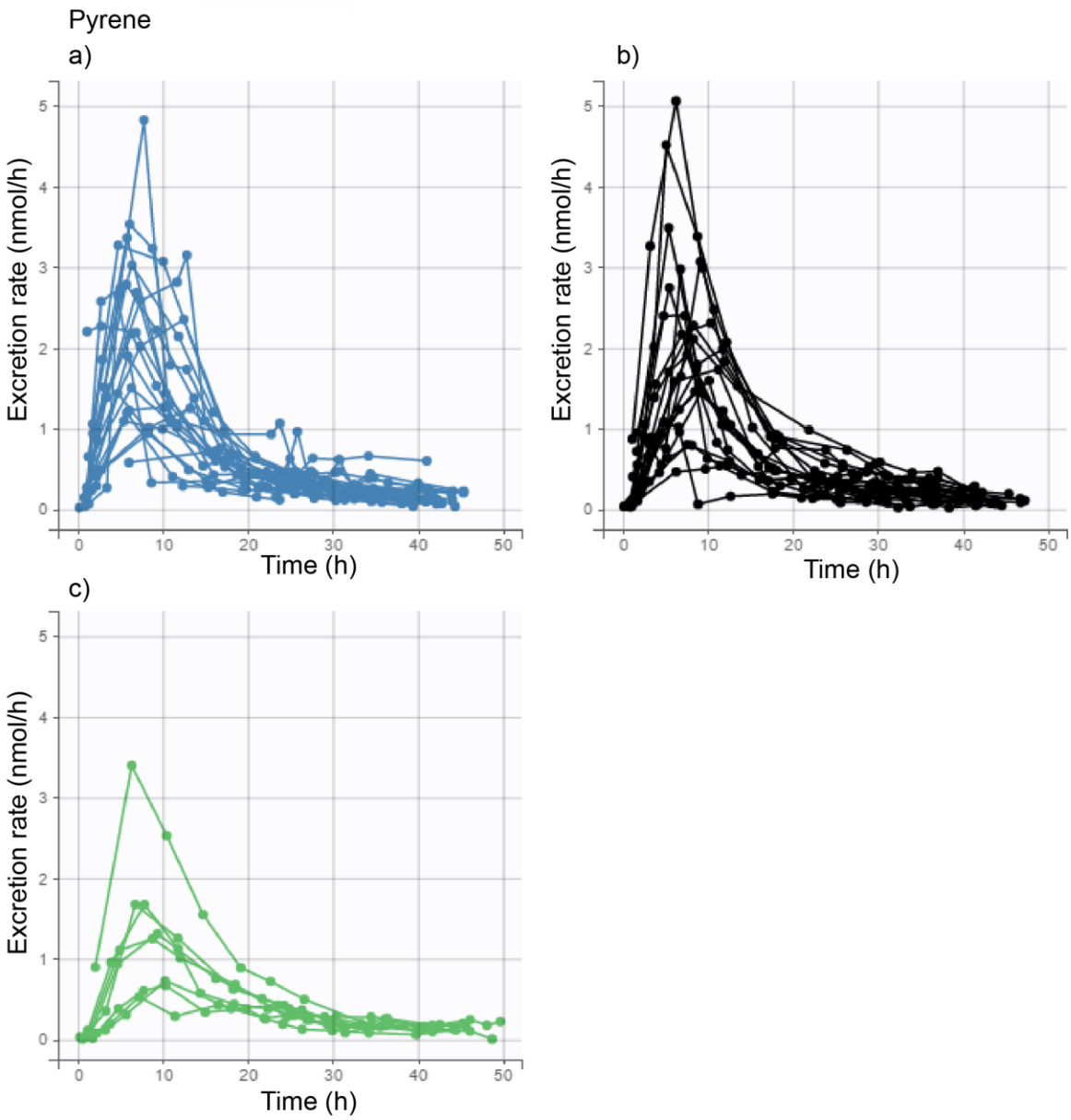


Figure S7. Excretion curves for oxybenzone by *FLG* genotype. Each curve represents an individual. Excretion rates of a) FLG null (blue) carriers, b) wt carriers with CNV20–22 (black) and c) wt carriers with CNV23–24 (green).



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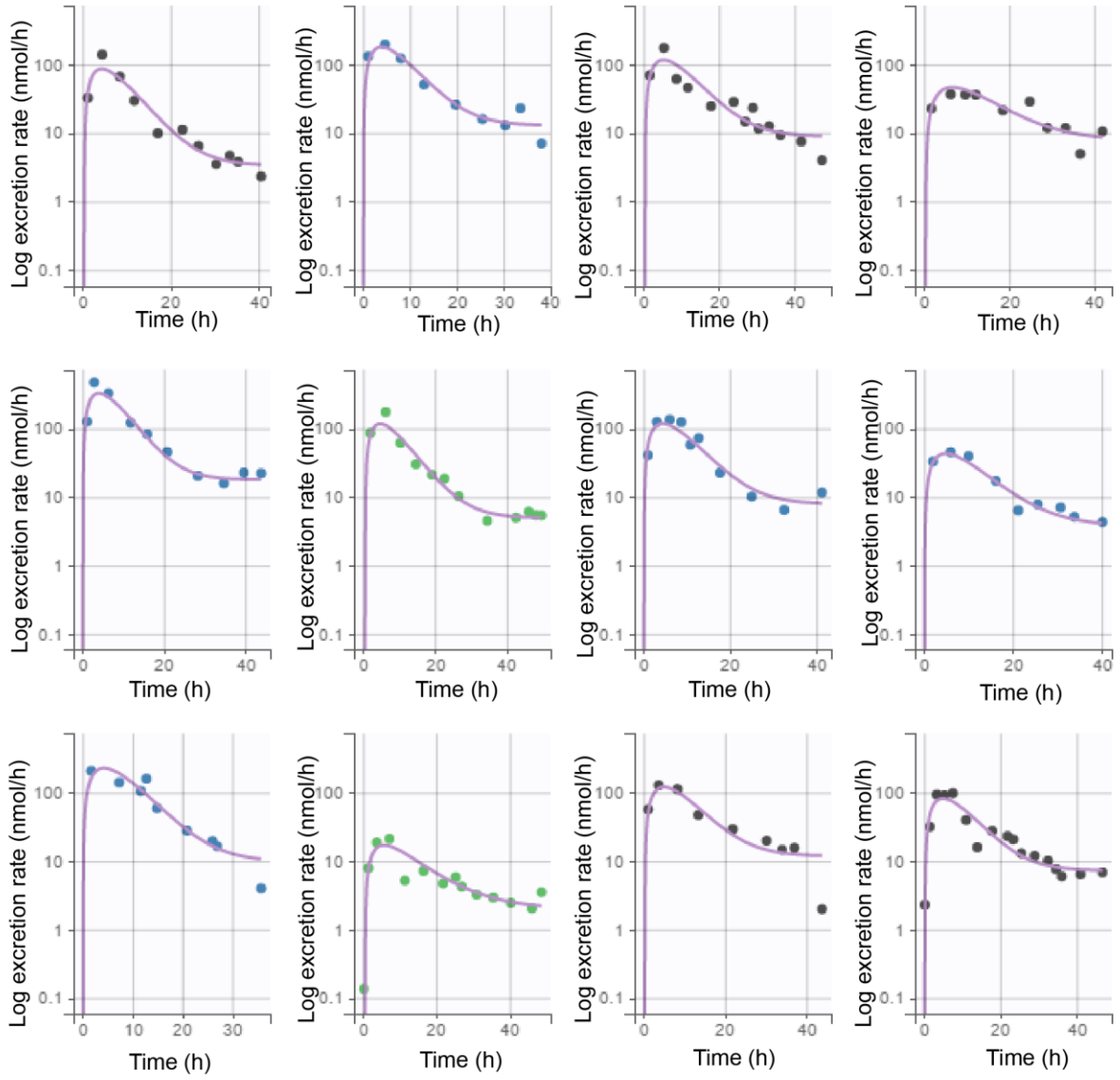

Figure S9. Examples of individual fits of the toxicokinetic model (purple line) to the excretion rate data (dots) for pyrimethanil. Blue = *FLG* null, black = wt CNV 20–22, green = wt CNV 23–24.