



Supplementary data

(A)

24 hpf - Experiment CBZ/E-CBZ	#	Body parts indistinguishable	Deviation tail	Oedema		Cardiovascular system	Malformation head
			Tissue deviation	Pericard	Yolk	Malformation heart	Deviating shape of head
EMS	1						
	2						
EMS 0.5% DMSO	1						
	2	1/20					
EMS 1% DMSO	1						
	2					1/20	1/20
CBZ 31.25 $\mu$ M	1						
	2				1/20		
CBZ 85 $\mu$ M	1						
	2		1/20	1/20			
CBZ 250 $\mu$ M	1						
	2				1/20		
E-CBZ 250 $\mu$ M	1						
	2						

(B)

48 hpf - Experiment CBZ/E-CBZ	#	Body parts unrecognizable	Hatching	Deviation tail		Malformation head	
				Curve	Tissue deviation	Deviating shape of head	Deviation eye
EMS	1		2/20				
	2		4/20				
EMS 0.5% DMSO	1		3/20				
	2	1/20	10/20			1/20	
EMS 1% DMSO	1		3/20				
	2		14/20				
CBZ 31.25 $\mu$ M	1		7/20				
	2		12/20				
CBZ 85 $\mu$ M	1		7/20		1/20		
	2		2/20 ***		2/20		
CBZ 250 $\mu$ M	1		1/20				
	2		9/20	2/20	1/20		1/20
E-CBZ 250 $\mu$ M	1		8/20		1/20		
	2		15/20				

(C)

72 hpf - Experiment CBZ/E-CBZ	#	Coagulation	Body parts indistinguishable	No hatching	Deviation tail		Oedema	Malformation head		Swim bladder not inflated
					Curve	Tissue deviation		Deviating shape of head	Deviation eye	
EMS	1				1/20	1/20		1/20		13/20
	2									10/20
EMS 0.5% DMSO	1									11/20
	2		1/20		1/20			1/20	2/20	9/20
EMS 1% DMSO	1									9/20
	2					1/20		1/20		8/20
CBZ 31.25 $\mu$ M	1				1/20					10/20
	2									9/20
CBZ 85 $\mu$ M	1				1/20					10/20
	2	1/20				1/19			2/19	6/19
CBZ 250 $\mu$ M	1			7/20 **		1/20				18/20 *
	2			8/20 **		3/20	3/20		1/20	12/20
E-CBZ 250 $\mu$ M	1									9/20
	2							1/20	1/20	6/20

(D)

96 hpf - Experiment CBZ/E-CBZ	#	Coagulation	No hatching	Deviation tail		Oedema Pericard	Oedema Yolk	Malformation head		Deviating pigmentation	Swim bladder not inflated
				Curve	Tissue deviation			Deviating shape of head	Deviation eye		
EMS	1			1/20	1/20			2/20			6/20
	2										4/20
EMS 0.5% DMSO	1										7/20
	2		1/20			1/20	1/20	1/20	1/20		3/20
EMS 1% DMSO	1										8/20
	2										5/20
CBZ 31.25 µM	1										6/20
	2				1/20						4/20
CBZ 85 µM	1			1/20	2/20			2/20			9/20
	2	1/20		1/19	3/19			2/19			5/19
CBZ 250 µM	1		5/20 *	1/20	5/20 *			5/20 *		13/20 ****	16/20 **
	2		4/20	1/20	4/20	2/20	1/20	2/20		15/20 ****	8/20
E-CBZ 250 µM	1										10/20
	2			1/20	1/20			2/20	1/20		4/20

**Figure S1.**(A): CBZ/E-CBZ morphological replicates – Time point 24 hpf, (B): CBZ/E-CBZ morphological replicates – Time point 48 hpf; (C): CBZ/E-CBZ morphological replicates – Time point 72 hpf; (D): CBZ/E-CBZ morphological replicates – Time point 96 hpf

(A)

48 hpf - Experiment PHE/HPPH	#	Hatching
EMS	1	10/20
	2	9/20
EMS 0.5% DMSO	1	13/20
	2	13/20
PHE 31.25 µM	1	9/20
	2	11/20
PHE 85 µM	1	6/20
	2	10/20
PHE 250 µM	1	0/20 ****
	2	4/20 **
HPPH 250 µM	1	15/20
	2	15/20

(B)

72 hpf - Experiment PHE/HPPH	#	No hatching	Deviation tail		Malformation head Deviation eye	Deviating pigmentation	Swim bladder not inflated
			Curve	Tissue deviation			
EMS	1						10/20
	2						7/20
EMS 0.5% DMSO	1				1/20		10/20
	2		1/20				7/20
PHE 31.25 µM	1					3/20	13/20
	2						10/20
PHE 85 µM	1			1/20		2/20	13/20
	2		3/20	3/20		4/20	13/20
PHE 250 µM	1	4/20	1/20	1/20	3/20	2/20	14/20
	2	1/20			4/20	5/20 *	17/20 **
HPPH 250 µM	1				1/20		11/20
	2		1/20		1/20		10/20

(C)

96 hpf - Experiment PHE/HPPH	#	No hatching	Deviation tail		Oedema		Malformation head		Deviating pigmentation	Swim bladder not inflated
			Curve	Tissue deviation	Head	Pericard	Deviating shape of head	Deviating eye		
EMS	1								1/20	4/20
	2									4/20
EMS 0.5% DMSO	1			1/20			1/20		1/20	5/20
	2		2/20				1/20			1/20
PHE 31.25 µM	1							2/20	3/20	9/20
	2								4/20	9/20 **
PHE 85 µM	1			4/20			3/20	2/20	1/20	11/20
	2		5/20	1/20			2/20	3/20	2/20	12/20 ***
PHE 250 µM	1	1/20	1/20	1/20		1/20	2/20		3/20	18/20 ****
	2	1/20	3/20		1/20		1/20	4/20	5/20 *	14/20 ****
HPPH 250 µM	1			1/20			1/20			5/20
	2			1/20						6/20

**Figure S2.**(A): PHE/HHPH morphological replicates – Time point 48 hpf; (B): PHE/HHPH morphological replicates – Time point 72 hpf; (C): PHE/HHPH morphological replicates – Time point 96 hpf

**Table S1.** List of molar and mass concentrations used for the different experiments per compounds. CBZ: carbamazepine; E-CBZ: carbamazepine-10,11-epoxide; PHE: phenytoin; HPPH: 5-(4-hydroxyphenyl)-5-phenylhydantoin; LAMO: lamotrigine

Compound	Molar concentration (µM)	Mass concentration	Samples
CBZ	0.02	3.5 ng/ml	LLOQ
	0.05	12.5 ng/ml	Standard curve - Quality control (Low)
	1.06	250 ng/ml	Quality control (Medium)
	10.5	2.5 µg/ml	Standard curve - Quality control (High)
	31.25	7.38 µg/ml	Samples – embryo exposure
	85	20 µg/ml	Samples – embryo exposure
	100	23.62 µg/ml	Samples – microsomes exposure
	250	59 µg/ml	Samples – embryo exposure
E-CBZ	0.002	0.5 ng/ml	Standard curve
	0.006	1.5 ng/ml	Quality control (Low) - LLOQ
	0.08	20 ng/ml	Quality control (Medium)
	0.3	75 ng/ml	Quality control (High)
	1	250 ng/ml	Quality control (Very high)
	1.59	400 ng/ml	Standard curve
	250	63 µg/ml	Samples – embryo exposure
PHE	0.01	1.75 ng/ml	LLOQ
	0.05	12.5 ng/ml	Standard curve - Quality control (Low)
	1.0	250 ng/ml	Quality control (Medium)
	10	2.5 µg/ml	Standard curve - Quality control (High)
	31.25	7.88 µg/ml	Samples – embryo exposure
	85	21.4 µg/ml	Samples – embryo exposure
	100	25.23 µg/ml	Samples – microsomes exposure
	250	63 µg/ml	Samples – embryo exposure
HPPH	0.007	2 ng/ml	Standard curve
	0.02	6 ng/ml	Quality control (Low) - LLOQ
	0.3	80 ng/ml	Quality control (Medium)
	1.12	300 ng/ml	Quality control (High)
	3.73	1000 ng/ml	Quality control (Very high)
	5.97	1600 ng/ml	Standard curve
	250	67 µg/ml	Samples – embryo exposure
LAMO	0.39	100 ng/ml	Samples – internal standard

Time point	Parameters																												
24 hpf	Pre-processing				separate deviations of morphology																								
	Coagulation	body parts indistinguishable	body parts unrecognizable	no hatching	deviation tail		Oedema				blood accumulation					Malformation yolk	Spilt yolk + extension	Cardiovascular system			Malformation head				deviating pigmentation	Non-detachment tail			
elbow					Curve	tissue deviation	head	Pericard	Yolk	Yolk extension /tail	tail	head	heart	Yolk	Yolk extension/tail			no blood circulation in heart	no blood circulation in tail	disturbed blood circulation in tail	heartbeat absent	deviating shape of head	deviation ear	deviation mouth			deviation eye		
48 hpf	Pre-processing				separate deviations of morphology																								
	Coagulation	body parts indistinguishable	body parts unrecognizable	Hatching	deviation tail		Oedema				blood accumulation					Malformation yolk	Spilt yolk + extension	Cardiovascular system			Malformation head				deviating pigmentation	Non-detachment tail			
elbow					Curve	tissue deviation	head	Pericard	Yolk	Yolk extension /tail	tail	head	heart	Yolk	Yolk extension/tail			no blood circulation in heart	no blood circulation in tail	disturbed blood circulation in tail	heartbeat absent	deviating shape of head	deviation ear	deviation mouth			deviation eye		
72 hpf	Pre-processing				separate deviations of morphology																								
	Coagulation	body parts indistinguishable	body parts unrecognizable	no hatching	deviation tail		Oedema				blood accumulation					Malformation pectoral fin				Malformation yolk	Cardiovascular system			Malformation head				deviating pigmentation	Non-detachment tail
elbow					Curve	tissue deviation	head	Pericard	Yolk	Yolk extension/ tail	tail	head	heart	Yolk	Yolk extension/tail	missing fin (left)	missing fin (right)	curved fin (left)	curved fin (right)		no blood circulation in tail	disturbed blood circulation in tail	heartbeat absent	deviating shape of head	deviation ear	deviation mouth	deviation eye		
96 hpf	Pre-processing				separate deviations of morphology																								
	Coagulation	body parts indistinguishable	body parts unrecognizable	no hatching	deviation tail		Oedema				blood accumulation					Malformation pectoral fin				Malformation yolk	Cardiovascular system			Malformation head				deviating pigmentation	Non-detachment tail
elbow					Curve	tissue deviation	head	Pericard	Yolk	Yolk extension/ tail	tail	head	heart	Yolk	Yolk extension/tail	missing fin (left)	missing fin (right)	curved fin (left)	curved fin (right)		no blood circulation in tail	disturbed blood circulation in tail	heartbeat absent	deviating shape of head	deviation ear	deviation mouth	deviation eye		
120 hpf	Pre-processing				separate deviations of morphology																								
	Coagulation	body parts indistinguishable	body parts unrecognizable	no hatching	deviation tail		Oedema				blood accumulation					Malformation pectoral fin				Malformation yolk	Cardiovascular system			Malformation head				deviating pigmentation	Non-detachment tail
elbow					Curve	tissue deviation	head	Pericard	Yolk	Yolk extension/ tail	tail	head	heart	Yolk	Yolk extension/tail	missing fin (left)	missing fin (right)	curved fin (left)	curved fin (right)		no blood circulation in tail	disturbed blood circulation in tail	heartbeat absent	deviating shape of head	deviation ear	deviation mouth	deviation eye		