Supplementary data for the article:

Savić, N. D.; Milivojevic, D. R.; Glišić, B. D.; Ilic-Tomic, T.; Veselinovic, J.; Pavic, A.; Vasiljevic, B.; Nikodinovic-Runic, J.; Djuran, M. I. A Comparative Antimicrobial and Toxicological Study of Gold(III) and Silver(i) Complexes with Aromatic Nitrogen-Containing Heterocycles: Synergistic Activity and Improved Selectivity Index of Au(III)/Ag(i) Complexes Mixture. *RSC Advances* **2016**, *6* (16), 13193–13206. https://doi.org/10.1039/c5ra26002g

A comparative antimicrobial and toxicological study of gold(III) and silver(I) complexes with aromatic nitrogen-containing heterocycles: Synergistic activity and improved selectivity index of Au(III)/Ag(I) complexes mixture

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Abstract

Five aromatic nitrogen-containing heterocycles, pyridazine (pydz, **1**), pyrimidine (pm, **2**), pyrazine (pz, **3**), quinoxaline (qx, **4**) and phenazine (phz, **5**) have been used for the synthesis of gold(III) and silver(I) complexes. In contrast to the mononuclear **Au1-5** complexes all having square-planar geometry, the corresponding **Ag1-5** complexes have been found to be polynuclear and of different geometries. Complexes **Au1-5** and **Ag1-5**, along with $K[AuCl_4]$, $AgNO_3$ and N-heterocyclic ligands used for their synthesis, were evaluated by *in vitro* antimicrobial studies against a panel of microbial strains that lead to many skin and soft tissue, respiratory, wound and nosocomial infections. All tested complexes exhibited excellent to good antibacterial activity with minimal inhibitory (MIC) values in the range of 2.5 to 100 μg mL^{-1} against the investigated strains. The complexes were particularly efficient against pathogenic *Pseudomonas aeruginosa* (MIC = 2.5–30 μg mL^{-1}) and had a marked ability to disrupt clinically relevant biofilms of strains with high inherent resistance to antibiotics. Moreover, the **Au1-4** and **Ag1-5** complexes exhibited pronounced ability to competitively intercalate double stranded bacterial genomic DNA of *P. aeruginosa*, which was demonstrated by gel electrophoresis techniques and supported by molecular docking into the DNA major groove. Antiproliferative effect on the normal human lung fibroblast cell line MRC5 has also been evaluated in order to determine therapeutic potential of **Au1-5** and **Ag1-5** complexes. Since the investigated gold(III) complexes showed much lower negative effects on the viability of the MRC5 cell line than their silver(I) analogues and slightly lower antimicrobial activity against the investigated strains, the combination approach to improve their pharamacological profiles was applied. Synergistic antimicrobial effect and the selectivity index of 10 were achieved for the selected gold(III)/silver(I) complexes mixtures, as well as higher *P. aeruginosa* PAO1 biofilm disruption activity, and improved toxicity profile towards zebrafish embryos, in

comparison to the single complexes. To the best of our knowledge, this is the first report on synergistic activity of gold(III)/silver(I) complexes mixtures and it could have an impact on development of new combination therapy methods for the treatment of multi-resistant bacterial infections.

Keywords: Silver(I) complexes; Gold(III) complexes; Antibacterial activity; Cytotoxicity; *Danio rerio*; Synergism

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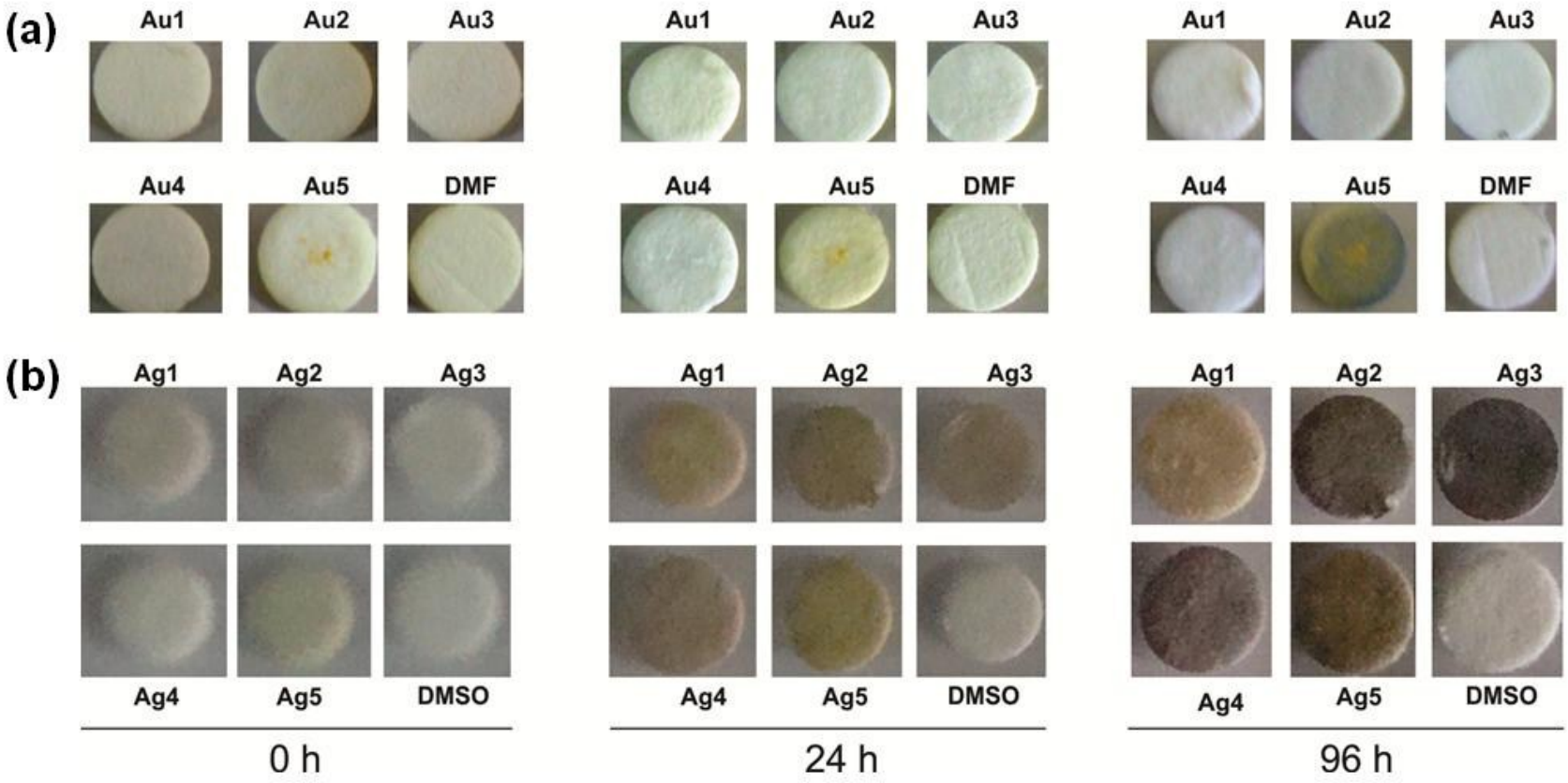

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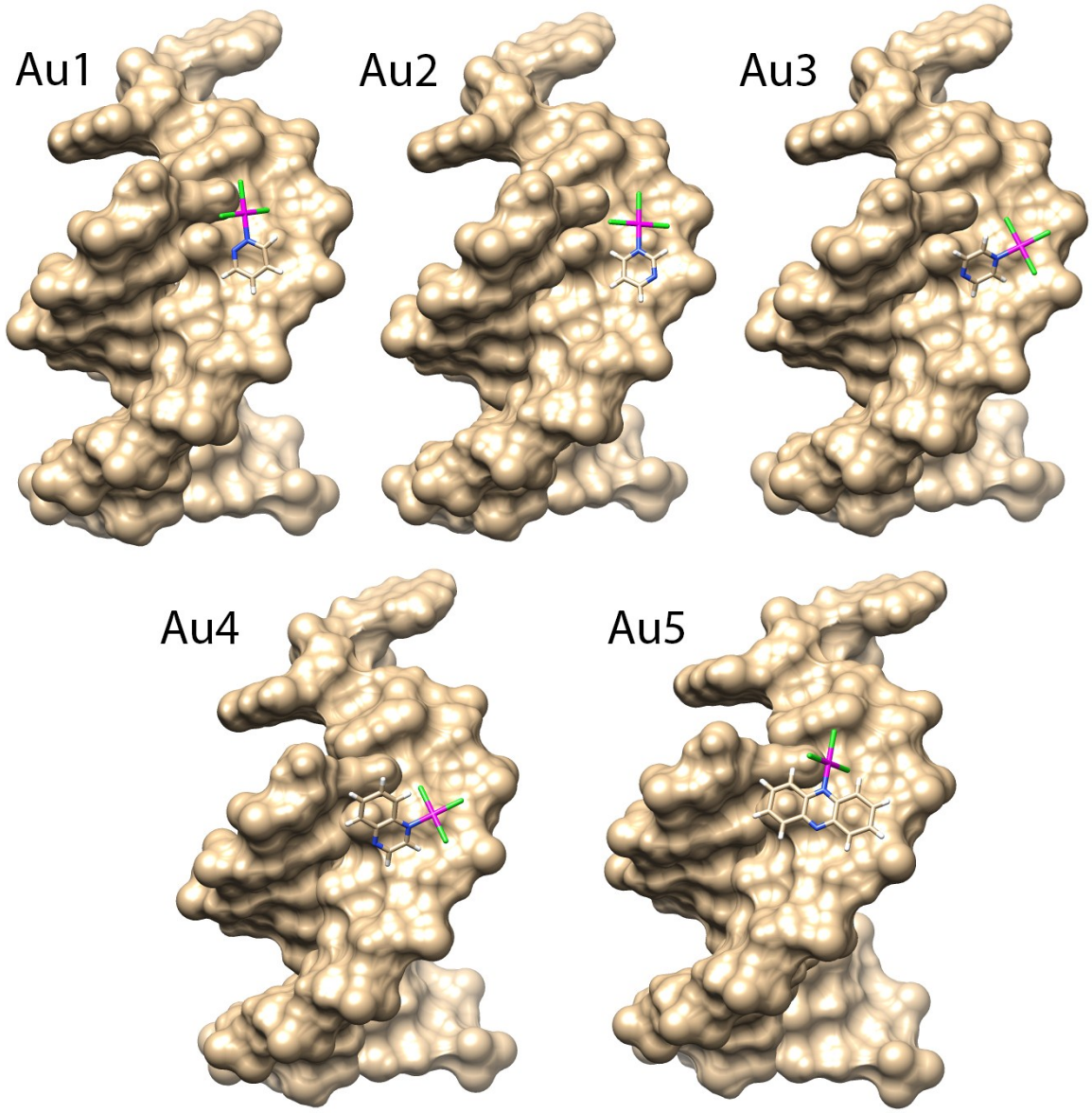

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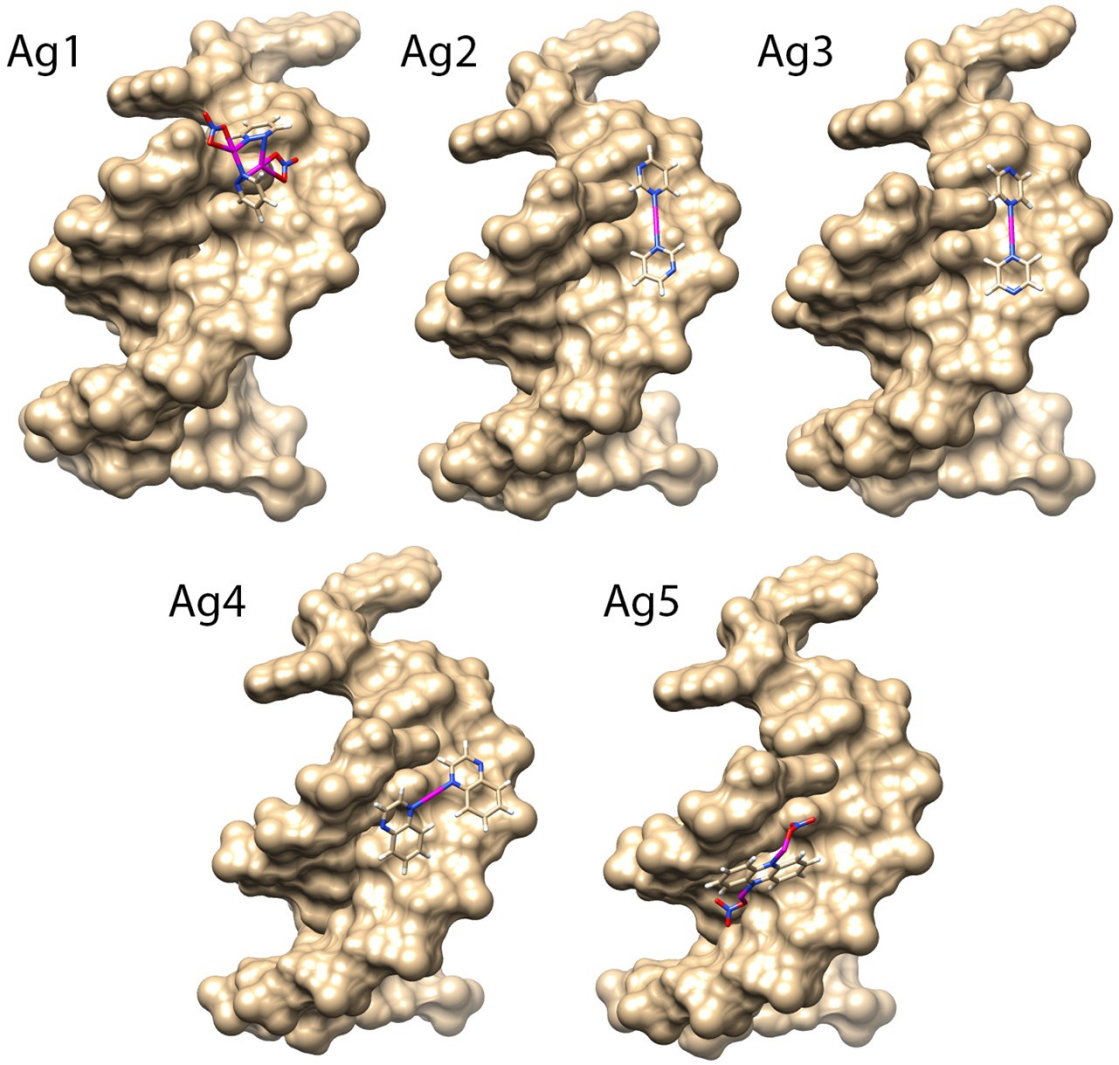

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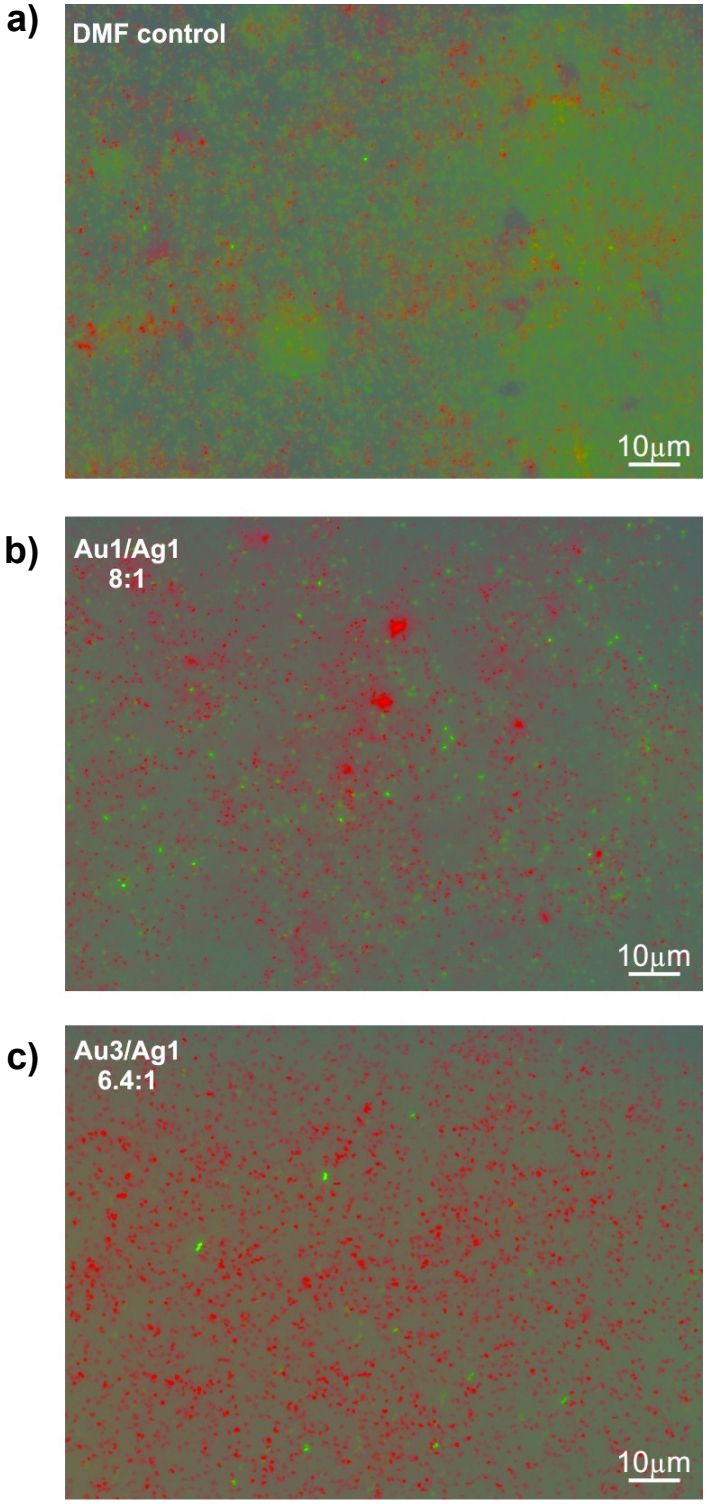

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Table S1 Comparison of MIC values (μg mL^{-1}) of the investigated gold(III) and silver(I) compounds in different incubation media

	***Pseudomonas aeruginosa* PAO1**		
Complex	***LB***	No serum	Serum
Au1	10 ± 1	10 ± 0.3	20 ± 0.8
Ag1	2.5 ± 0.1	5 ± 0.1	5 ± 0.1
Au2	15 ± 1	15.6 ± 1	31.2 ± 1
Ag2	2.5 ± 0.1	5 ± 0.1	10 ± 0.1
Au3	8 ± 0.5	15.6 ± 0.5	15.6 ± 1
Ag3	7.8 ± 0.5	10 ± 0.4	10 ± 0.6
Au4	12.5 ± 0.5	15.6 ± 0.5	31.2 ± 0.8
Ag4	15.6 ± 0.5	15.6 ± 0.5	15.6 ± 0.5
Au5	30 ± 4	62.5 ± 6	62.5 ± 6
Ag5	7.8 ± 4	15.6 ± 4	15.6 ± 4
$K[AuCl_4]$	8 ± 0.5	15.6 ± 0.5	31.2 ± 1
$AgNO_3$	3.1 ± 0.1	5 ± 0.1	5 ± 0.1

Table S2 Effect percentages for abnormal morphological characteristics evaluated in the zebrafish teratogenicty assay at 114 hpf

Compound concentration	Dead embryos[a]	Teratogenic embryos[a]	Normal embryos[a]	Growth retardation[b]	Notochord[b]	Eyes[b]	Otoliths[b]	Pericardial edema[b]	Yolk edema[b]	Heart beat[b]	Blood circulation[b]	Unhatched[b]	Head malformation[c]	Skeletal deformities[c]
Ag1														
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-
10 µg/mL	95.83	4.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00
5 µg/mL	25.00	75.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00
Au1														
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	58.33	41.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
10 µg/mL	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
5 µg/mL	0.00	8.33	91.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au3														
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	
25 µg/mL	95.83	4.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	n.d.	n.d.
10 µg/mL	62.50	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	n.d.	n.d.
5 µg/mL	8.33	12.50	79.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.64
Au1/Ag1 8:1														
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	62.50	0.00	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au3/Ag1 6.4:1														
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	62.50	0.00	37.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Abbreviation used: (hpf) hours post fertilization; (-) data not available due to 100% mortality; (n.d.) not detected.
[a] Percentage of mortality based on all eggs.
[b] Percentage of teratogenic effect based on all alive embryos at the time of assessment.
[c] Percentage of teratogenic effect based on all alive hatched embryos at the time of assessment.

Table S3 Lethal and teratogenic effects observed in zebrafish (*Danio rerio*) embryos at different hours post fertilization (hpf)

Category	Developmental endpoints	Exposure time (hpf)			
		24	48	72	96 /114
Lethal effect	Egg coagulation[a]	•	•	•	•
	No somite formation	•	•	•	•
	Tail not detached	•	•	•	•
	No heartbeat		•	•	•
Teratogenic effect	Malformation of head	•	•	•	•
	Malformation of eyes[b]	•	•	•	•
	Malformation of sacculi/otoliths[c]	•	•	•	•
	Malformation of chorda	•	•	•	•
	Malformation of tail[d]	•	•	•	•
	Scoliosis	•	•	•	•
	Heart beat frequency		•	•	•
	Blood circulation		•	•	•
	Pericardial edema	•	•	•	•
	Yolk edema	•	•	•	•
	Yolk deformation	•	•	•	•
	Growth retardation[e]	•	•	•	•

[a] No clear organs structure is recognized.
[b] Malformation of eyes was recorded for the retardation in eye development and abnormality in shape and size.
[c] Presence of no, one or more than two otoliths per sacculus, as well as reduction and enlargement of otoliths and/or sacculi (otic vesicles).
[d] Tail malformation was recorded when the tail was bent, twisted or shorter than to control embryos as assessed by optical comparation.
[e] Growth retardation was recorded by comparing with the control embryos in development or size (before hatching, at 24 hpf and 48 hpf) or in a body length (after hatching, at and onwards 72 hpf) using by optical comparation using a inverted microscope (CKX41; Olympus, Tokyo, Japan).