pesticides chlorpyrifos and endosulfan, and combinations of these. NMR and MS metabolic profiling, microarray and RT-qPCR analysis were used to identify novel biomarkers. The cytotoxicity evaluation of the individual contaminants showed the greatest cytotoxic effects with phenanthrene, followed by benzo(a)pyrene and endosulfan, while chlorpyrifos had no cytotoxic effect. Lipidomic, microarray and RT-qPCR analyses suggested perturbation of lipid metabolism, as well as endocrine disruption. The pesticides gave the strongest responses, despite having less effect on cell viability than the PAHs. Only weak metabolic perturbation was detected in PAH-exposed hepatocytes. Chlorpyrifos suppressed the synthesis of unsaturated fatty acids. Endosulfan affected steroid hormone synthesis, while benzo(a)pyrene disturbed vitamin D3 metabolism. The primary mixture effect was additive, although at high concentrations the pesticides acted in a synergistic fashion to decrease cell viability and down-regulate CYP3A and FABP4 transcription. This work highlights the usefulness of using cell cultures, 'omics techniques and multivariate data analysis to investigate modes of action of single contaminants and interactions within mixtures of contaminants associated with novel feed ingredients to farmed fish.

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P-4.32
Toxicology and risk assessment of chemical mixtures

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Evaluating potential health risks posed by exposures to multiple chemicals is challenging for toxicology research and risk assessment (RA). Problem formulation is complex and mixtures RA frameworks vary greatly among agencies. This study (1) describes RA approaches for chemical mixtures; (2) characterizes differences in RA frameworks; and (3) assesses regulatory acceptance of these frameworks with a focus on food mixtures. Reviews were completed for mixture RA paradigms used by US federal agencies, nonprofit organizations (WHO, IPCS) and international agencies (EU). There are significant overlaps and differences between paradigms and there appears to be no single unified approach. Considering the current challenges in food mixtures, a hypothetical case study was conducted using a tiered screening approach and hazard index method to evaluate the effects of mixtures in foods. A hypothetical new bean product is being considered to replace pinto beans for a food program. Component levels of the following differ between old beans (OB) and new beans (NB) (assuming a 10% decrease): cadmium, deltamethrin, cyfluthrin; and bisphenol A levels are the same in OB and NB. Non-cancer health effects following chronic oral exposure were evaluated. First tier provided a crude filter using the most recent and conservative health reference values for mixture components. Some assumptions in the first tier were refined in the second tier. Components were grouped based on health effects and biomonitoring data were used to estimate exposure in the second tier. Results indicate that tiered approach is a useful way of rapidly screening the effects of chemical mixtures.

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P-4.33
Effects of copper and lead singly and with mixture of chitosan on some sera parameters of *Clarias gariepinus*

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Effects of copper and lead applied singly and in mixture with chitosan on sera aspartate aminotransferase (AST), alanin aminotransferase (ALT), glucose, total protein and cholesterol levels of *Clarias gariepinus* were studied after exposing the animals to 5.0 ppm Cu, 1.0 ppm Pb and 75 ppm chitosan concentrations of the metal over 1, 7 and 15 days. Sera parameters were measured using an auto analyzer and statistical evaluation of the experimental data was carried out by Variance Analysis and Student Newman Keul's Procedure (SNK).

Abundant biosorbants such as chitin and chitosan are known to form stable complexes with many metal ions. Chitin is widely distributed in nature, especially in the exoskeletons of marine invertebrates such as prawn, crab and lobster whereas its derivative chitosan has reactive amino groups which forms complexes between metal ions and the polymer chain. Affectivity of chitosan in binding copper and lead is known to increase with increasing deacetylation times.

No mortality was observed during the experiments. AST, ALT, glucose, total protein and glucose levels changed with the concentration of metal and with exposure periods. Sera glucose levels increased with increasing exposure periods at the determined concentrations of metals which might be due to stimulation of glycogenolysis in muscle and liver by affecting carbohydrate metabolism depending on increased energy requirement under the effect of metals. Sera AST and ALT levels also increased with increasing exposure periods which can be explained by the tissue damage under the effect of metals.

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P-4.34
In vitro cytotoxic effects, antioxidant capacity and phytochemical evaluations of extracts of *Clausena anisata*, *Peltophorum africanuum* and *Zanthoxylum capense*

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Extracts of *Clausena anisata* (CA), *Peltophorum africanuum* (PA) and *Zanthoxylum capense* (ZC) are used in South Africa to treat infections, inflammation and for pain relief. Preparations are usually taken orally in the form of decoction (tea) or infusions. Ingestion of products with toxic components can potentially impact negatively on the health of consumers, both in the short and long term.

Acetone extracts were prepared from the dried leaves of CA, PA and ZC using standard methods. The antioxidant activities of the extracts were done using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays. Cytotoxic effects were evaluated using both 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) and