Toxicological Analysis of PAH-rich Soot Extracts from PUV Exhausts Using the Zebrafish Embryo Assay

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RESEARCH ARTICLE

Abstract

Background & Objective: Polycyclic aromatic hydrocarbons (PAH) are among the most toxic environmental pollutants and carcinogens known. Vehicular emissions resulting from rampant utilization of fossil fuel and other petrogenic resources have increased PAH levels in the environment. Renewed interests among scientists have emerged to determine the ecotoxicological potencies of PAH-rich soots derived from incomplete combustion of fuels used by the transport sector. Thus, the present study was carried out to investigate the kinds, concentrations, and embryotoxicity potential of the two categories of PAH-laden soot extracts obtained from public utility vehicle (PUV) tailpipes.

Methodology: Two categories of soot from pure diesel and diesel-biodiesel blend-fuelled vehicles, were collected from tailpipes of public utility vehicles (PUVs) stationed in several terminals along Quezon City, Philippines. All samples were subjected to solid-phase extraction clean-up, followed by GC/MS spectrometric analyses to determine the kinds and concentrations of PAHs. The soot extracts were also prepared and used for the zebrafish embryo assay.

Results & Conclusions: Results showed that both types of soot samples contained comparable levels of environmentally relevant PAHs. The diesel extracts contained 10 PAHs that registered higher average levels compared to only 4 in the fuel blend extracts. All undiluted extracts, whether from diesel or fuel blend soot, were embryotoxic to zebrafish embryos (ie., egg coagulation within 12 to 24 hrs). Extracts from both types of soot showed decreasing levels of toxicities upon dilution, in terms of the number of abnormalities and lethal endpoints observed. Overall, there was no marked difference between the two types of soot extracts in terms of toxicities and PAH kinds and levels. The results of the study could provide a benchmark for the development of a rapid-response model for predicting teratogenic potential of combustion-derived soots in a broad range of vertebrates.

Keywords: *biodiesel, diesel, embryotoxicity, PAHs, PUV-exhausts, soot extract, zebrafish*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) belong to a group of organic chemical compounds that are characterized by two or more fused aromatic rings. These compounds occur either as natural components of organic materials such as coal, coal tars, wood, oil, tobacco, and petroleum (petrogenic PAHs) or as byproducts of combustion (pyrogenic PAHs) [1,2]. However, much of the current environmental PAH burden are in the form of complex mixtures of PAH-rich soots derived from incomplete combustion of fuels used by the industrial and transport sector [3]. Higher levels of PAHs are being reported in urban atmospheres due to the increased amounts of gas and oil burned in these sites [4,5,6]. In Manila alone, 84% of particulate emissions come from motor vehicles while the remaining 16% come from solid waste burning and industries. Furthermore, 70% of those motor vehicles come from the more than 200,000 diesel-powered utility vehicles such as jeepneys and the 170,000 gasoline-powered motorcycles and tricycles plying across the city [7].

PAHs also accumulate in the soil matrix, and levels are correlated with the extent of activities, such as mining,

transport, industries, oil refineries, ship docks, and agriculture [8,9]. From the soil, PAH contaminations reach the vegetation and ultimately spread in the entire food chain [10]. Several studies have also determined the levels of PAHs in bodies of water, such as rivers and bays as well as in drinking water. PAHs are transported in pathways through rain and runoff until they settle on sediments and marine organisms which then act as adsorption sites for PAHs to bioaccumulate [9].

The lower molecular weight PAHs (2-3 rings) are lighter. Consequently, they volatilize readily into the air or are degraded by sunlight and other chemicals in a matter of days or weeks. These lighter PAHs are less toxic to humans and are not carcinogenic compared to the heavier PAHs (4-6 rings). When compared to non-combustion sources, the combustion products, such as those from exhaust soots, contain greater proportion of the heavier PAHs due to the thermal fusion of aromatic rings [11]. These PAHs are not readily soluble in water, and possess lipophilic properties that enable them to adsorb to solid particles and to settle onto sediments at the bottom of lakes, rivers, or streams. PAHs are considered as persistent organic compounds that pose concern on both environmental and human health. Many studies have already established the mutagenic and carcinogenic properties of PAHs, especially benzo(a)pyrene, which is considered the most potent PAH [12-16]. For European countries, they recommend a standard of 0.25 ng/m3 of benzo(a)pyrene. In the Philippines, there are no currently imposed regulations for PAHs. This is probably due to the very limited studies on the analysis of PAH levels from various sources that are available [9]. Data on PAH in sediments were obtained but not from emission sources [17].

Many studies have already recognized the use of zebrafish embryos as a suitable vertebrate model for studying the teratogenic effects of chemical stressors [6,17-21]). The use of the zebrafish system ensures easy procurement, low cost, and a readily maintainable source of parent zebrafish. The usually large number of fully transparent and nonadherent zebrafish eggs upon spawning exhibit rapid development and offer several endpoints for a finer evaluation and characterization of developmental effects due to toxicants [17,22,23].

The present study was performed to achieve the following objectives: (1) to determine and compare the identity and levels of PAHs in diesel and fuel blend available in the Philippines during the study period (ie., 1% biodiesel in diesel) soot extracts collected from exhaust engines of PUVs (specifically jeepneys) plying some routes in Quezon City, Philippines, (2) to determine if PAH-laden soot extracts elicit teratogenic potential by causing alterations in zebrafish embryo development, and (3) to compare relative embryotoxicity of diesel and biodiesel soot extracts.

Methodology

Sample Collection

Particulate samples were collected from tailpipes of public utility vehicles (PUVs) stationed in some jeepney terminals along Quezon City, Philippines. Samples were differentiated according to the type of fuel used (diesel or biodiesel blend). The samples were placed in glass jars previously washed free of organic material. Samples were then stored under nitrogen gas, sealed with Teflon and kept at 4^{oC} until analysis.

Solid-Phase Extraction Cleanup

The cleanup procedure was adapted from Xie et al. [24]. Soot measuring 0.1 gram was weighed and extracted with 30 mL hexane:dichloromethane (DCM) (50:50) in an ultrasonic bath for 30 minutes. All trials were done in triplicate. The soot extracts were concentrated in a rotary evaporator to a volume of 1 mL. A 3 mL solid phase extraction (SPE) silica tube (Supelco) was first cleaned with 10 mL DCM followed by 10 mL hexane. The flow rate in the manifold was maintained at 1 mL/min. The 1 mL sample extract was loaded to the SPE column and the non-PAH fraction was discarded. 3 mL of 20% DCM in hexane was then used to elute the PAH fractions. Nitrogen blowdown was used to concentrate the final extract to 1 mL. The extracts were stored at 4^{oC} until analysis.

Gas Chromatography/Mass Spectrometry Analysis

The analyses of PAHs were carried via splitless mode on a Varian 4000 GC-MS. The injector temperature was set at 280^{oC}, transfer line at 280^{oC}, ion trap at 150^{oC}, and the ion source at 230^{oC}. The column temperature program was set as follows: initial temperature at 55^{oC}, hold for 1 minute; ramp of 30^{oC}/min to 140^{oC}; ramp of 5^{oC}/min to 240^{oC}, hold for 5 minutes; and ramp of 8^{oC}/min to 300^{oC}, hold for 12 minutes. PAHs were identified by the NIST software and validated using the retention times of standards. The quantification of PAHs was obtained by external calibration method.

Preparation of extracts for the zebrafish egg assay

Soot measuring 1.0 gram was weighed and extracted with 30 mL hexane:dichloromethane (DCM) (50:50) in an ultrasonic bath for 30 minutes. All trials were done in triplicate. The extracts were concentrated in a rotary evaporator to a volume of 1 mL. A 3 mL solid phase extraction (SPE) silica tube (Supelco) was first cleaned with 10 mL DCM followed by 10 mL hexane. The flow rate in the manifold was maintained at 1 mL/min. The 1 mL sample extract was loaded to the SPE column and the non-PAH fraction was discarded. 3 mL of 20% DCM in hexane was then used to elute the PAH fractions. The extracts were evaporated to near dryness and diluted to 10 mL of 1% DMSO and used for zebrafish assay. Grouping of the extracts was made in such a way that samples 1 to 10 (S1 to S10) came from diesel soot extracts while samples 11 to 20 (S11-S20) from biodiesel soot extracts.

Zebrafish Egg/Embryo Assay

Zebrafish adults were maintained in several aquaria with the following conditions: water temperature (26±.5 8C), hardness (379 mg/L CaCO3), conductivity (744 S), pH (7.36±.2) and dissolved oxygen (10.5±.5=95% saturation). Ammonia, nitrite, and nitrate were kept below detection limits (ammonia: 0–5 mg/L; nitrite: 0.025–1 mg/L; nitrate: 0–140 mg/L). The photoperiod was set at 12-h light and 12-h darkness. Fish were fed twice daily with TetraMin dry flakes (Tetra, Melle, Germany) alternatively with red mosquito larvae from uncontaminated sources.

The zebrafish eggs were obtained in an aquarium with a group of 25 male and female zebrafish adult at a ratio of 3:2 (males:females). On the evening before spawning was required, several rectangular mesh wire boxes with attached artificial plants were placed at the bottom of the aquarium to collect the eggs the following morning. Spawning was triggered once the light was turned on and was completed within 30 min.

The study used the static test design that is based on the German Standard DIN 38415-6 [25], Nagel [23] and the OECD- draft for the Fish Egg Test (FET) [26] with zebrafish (*Danio rerio*). Fertilized eggs (up to 3hpf or 128-cell stage) were collected and immediately placed on preliminary test solutions containing the individually labelled soot extracts (diesel S1-S10 and biodiesel S11 to S20) to ensure immediate exposure. After which, individual eggs were placed in 96-well plates containing the test solutions with the individual soot extracts. The test was done only if the fertilization rate per batch reached 80%.

Since all extracts were 100% embryotoxic to zebrafish eggs (i.e egg coagulation within 12-24 hours), dilutions (1:0, 1:1, 1:2, 1:3, and 1:4) were also prepared from the DMSO stock solution with reconstituted water (DIN, 2001). The reconstituted water alone without the chemical served as the negative control (mortality < 10%) while 1% DMSO was used as the solvent control. As a standard positive control, 2.0% ETOH solution (mortality > 10%) was used [22]. Test results are valid according to DIN 38415-6 criteria. Each chemical was tested in at least three independent replicates with twelve embryos per test concentration and controls. Exposure was carried out up to 72 hours post fertilization. After an exposure time of 48 h, lethal endpoints (coagulation of the embryo, nondetachment of tail, and non-detection of heart beat) and other developmental responses (pericardial and yolk sac edemas, reduced pigmentation, and delayed or no hatching) were monitored by digital microscope attached to computer monitor (MIC-D M01-BSW Version 1.06, Olympus Ltd). After hatching, the following parameters were also evaluated: edemas, spine malformation, and mortality. All fish care and experimental techniques were reviewed and approved by the University of the Philippines Manila/National Institute of Health Institute for Animal Care and Use Committee IACUC).

For the embryotoxic endpoints, the treatment means from each site were tested for normality using the Shapiro-Wilk W Test. Treatment means that met the parameter assumptions of normality and homogeneity were then compared with the control using One-Way Analysis of Variance (ANOVA), and this was followed by Dunnett's test (Microsoft Excel-Analyze It™). Values of $p < 0.05$ and $p < 0.01$ were used to indicate significant difference.

Results and Discussion

The present study represents one of a few studies on the toxicological potencies of PAH-laden soot extracts obtained from tailpipes of public utility vehicle (PUV) plying across busy streets in a metropolitan city. Previous studies have clearly shown that soot samples contain environmentally relevant PAH mixtures that can induce greater toxicity than those predicted by simple additive toxicity of component PAHs alone [13,27]. Despite the reported 5000 premature deaths per year due to atmospheric pollution in the Philippines [7], the country still lacks regulation concerning

PAH levels. As a result, the general public is still at high risk for developing respiratory and cardiovascular ailments due to exposure to exhaust-laden PAHs. While there has been improvement in policies, such as the recommendation to use a diesel-biodiesel blend (referred to as biodiesel, for brevity), such changes should be supported by comparative studies on toxic potentials between pure petrodiesel and biodiesel fuel.

Figure 1 shows the qualitative chromatogram which identifies the presence of the PAHs extracted from soot samples. Table 1 shows the summary of the ranges of values found on the samples. The predominance of phenanthrene, fluoranthene, and pyrene in the soot samples is consistent with literature [6,19,28]. However, higher molecular weight PAHs (chrysene, benzofluoranthene, indeno pyrene, and benzoperylene) had high levels also in the soot samples.

Figures 2 and 3 compare the average PAH levels in diesel and biodiesel soot samples. In terms of total PAH, Figure 2A shows comparable levels for both diesel and biodiesel samples. Figure 2B shows that, in terms of average levels of particular PAHs, ten of the fifteen identified PAHs had higher average levels for the diesel compared to the biodiesel samples. Four had higher average PAH levels for biodiesel compared to the diesel samples, with one (naphthalene) comparable for both types of samples. The presence of PAHs in both soot extracts as well as the relatively higher levels of PAHs associated with petrodiesel compared to biodiesel are in concordance with previous studies [12,13,29] have demonstrated that the reduction in hydrocarbon content in biodiesel is paralleled by the reduction of life cycle CO^2 emissions by 50-75%, compared to petrodiesel. Biodiesel is also shown to reduce most of the exhaust emissions when used in unmodified diesel engines [12,30]. This reduction depends also on the blend level with B20 (20% biodiesel, 80% diesel fuel) decreasing particulate matter (PM) by 10.1%, hydrocarbon (HC) by 21.1% and carbon monoxide (CO) by 11.0%. [31,32]. This property of

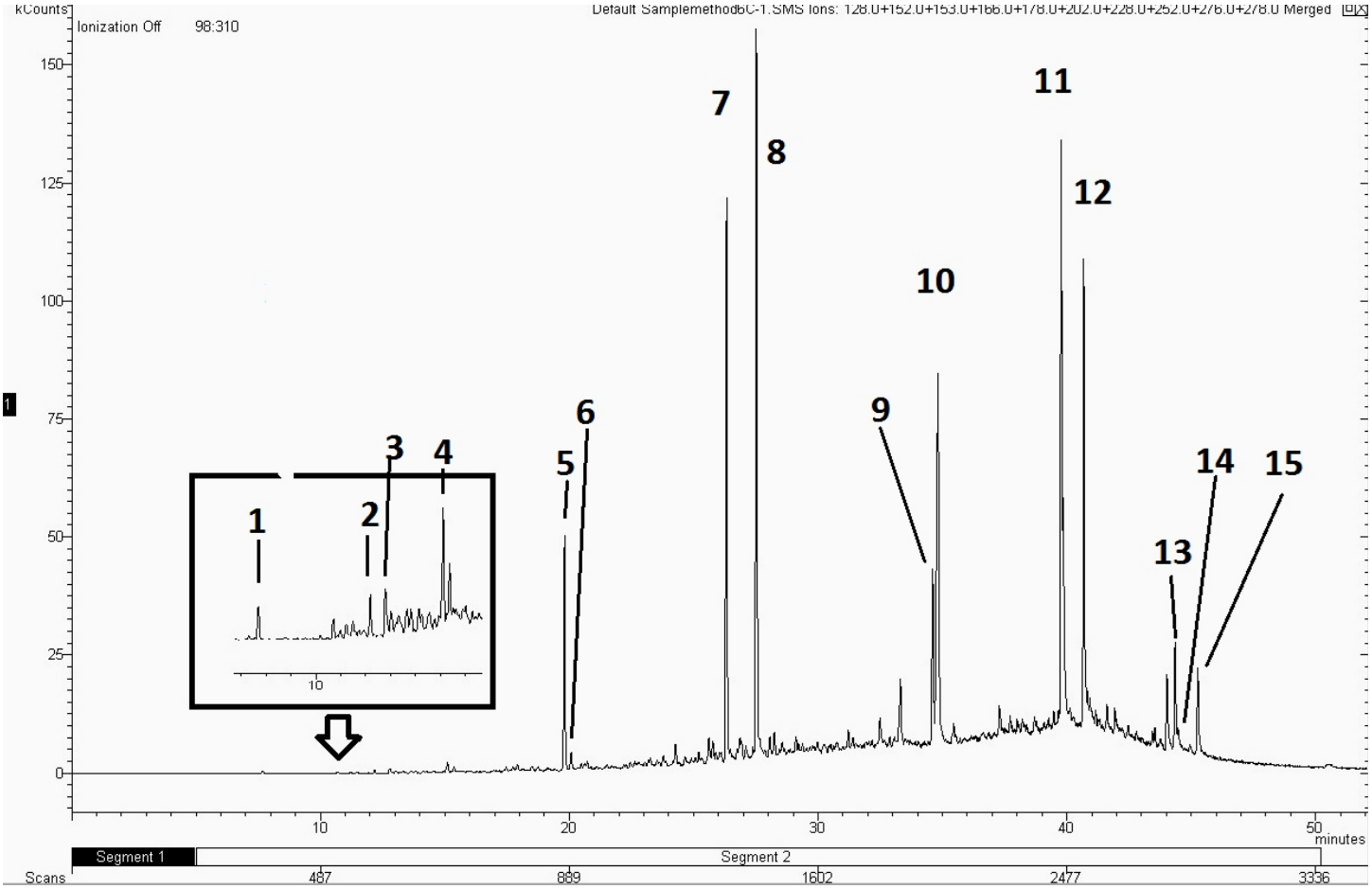

Figure 1. *GC/MS chromatogram showing the following PAHs from soot samples: (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzanthracene, (10) chrysene, (11) benzo(b)fluoranthene, (12) benzo(a)pyrene, (13) indenopyrene, (14) dibenzanthracene, (15) benzo(ghi)perylene*

Table 1. *Summary of PAH levels in the soot samples.*

PAH	DIESEL (N = 10)	BIODIESEL (N = 10)
	RANGE (µg/g)	RANGE (µg/g)
NAP napthalene	0.230-3.404	0.003-4.311
ANY acenaphthylene	0.230-2.283	0.013-1.887
ACP acenaphthene	0.085-1.776	ND
FLU fluorene	0.182-1.050	0.337-1.427
PHEN phenanthrene	1.069-13.950	0.706-13.681
ANT anthracene	0.171-1.005	0.315-1.516
FLT fluoranthene	1.337-27.061	0.655-20.947
PYR pyrene	2.147-30.815	0.884-27.116
BaA Benzo[a]anthracene	0.230-10.909	0.869-9.605
CHR chrysene	0.388-12.730	0.756-26.155
BbF Benzofluoranthene	4.275-54.609	0.497-36.186
BaP Benzo(a) pyrene	0.099-11.325	0.784-13.973
IPY Indeno pyrene	1.489-27.774	1.163-45.585
DBA dibenzoanthracene	0.847-7.451	2.384-3.841
BgP benzoperylene	0.335-21.226	0.557-26.056

biodiesel blend has attracted worldwide interest and made biodiesel a very suitable replacement for the traditional petrodiesel. However, all of these advantages are offset by the relatively higher levels of hazardous nitrogen oxides (up by 2-4%) forming smogs and some aldehydes in biodiesel exhausts as reported by [13].

Figures 3 to 4 relate the PAH levels in diesel and biodiesel soot extracts in terms of embryotoxicity results in the zebrafish egg assay. In Figure 4, samples S1 to S10 are diesel soot sample extracts, and samples S11 to S20 are biodiesel soot sample extracts. The samples were divided into two categories: The first is labelled "toxicity decr (meaning decreases) with dilution", which refers to major finding no. 2 below. The second is labelled "toxic (all dilutions)", which refers to major finding no. 3 below. It is to be noted that diesel and biodiesel samples can be found in both categories.

The major findings are as follows:

1. All undiluted samples (1:0) and those diluted to 50% (1:1) were 100% toxic to zebrafish embryos. All exposed embryos coagulated within 12-24 hours of exposure.
2. Starting with 1:2 down to 1:4 dilution, there was a decreasing severity on the embryotoxic and teratogenic effects observed in the zebrafish embryos. Furthermore, a characteristic dose-response relationship could be clearly seen. This was observed for samples S2, S4, S6, S7, S10, S11, S12, S13, S15, S18 and S20 (See Fig. 4B).
3. However, there were also samples namely *S1, *S3, *S5, *S8, *S9, *S14, *S16, *S17, and *S19 which caused 100% mortality in all tested dilution levels (1:0- 1:4) within 24 hours (See Fig. 4A).
4. The most common toxic effects include coagulation of embryos, growth retardation (rachischisis), no somite formation, undetachment of tail, underdeveloped head region, double yolk sac, enlarged and fused yolk sac, lower heart rate, yolk sac and pericardial edema, reduced pigmentation, and spinal malformations. (See Figs. 5-7). All embryotoxic effects were found to be statistically significant if compared to the negative and solvent control ($p<0.001$).
5. Normal development of zebrafish embryos (as described in Kimmel *et al.* 1995) was consistently observed in both the negative control (ISO artificial water) as well as the solvent control (1% DMSO) (See Figs. 5-7)
6. Positive control (2% ETOH) induced embryotoxic and teratogenic malformations in zebrafish embryos (See Figs. 5 and 6)

Figure 4B shows 12 of the 15 PAHs to have higher average levels in the 2nd category (toxic all dilutions) compared to the first. Also, in contrast to the diesel/biodiesel comparison

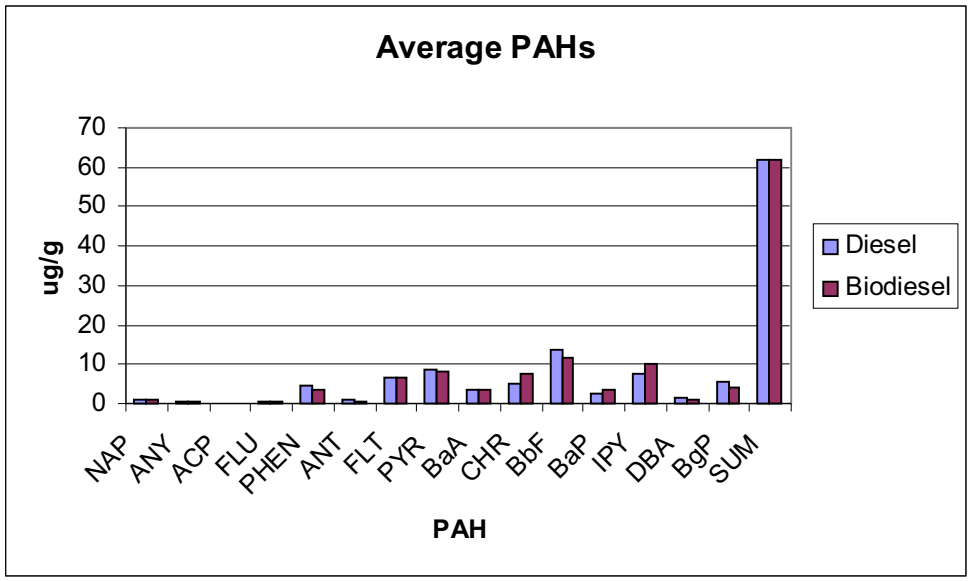

Figure 2A. *Comparable average levels of PAH in diesel and biodiesel exhaust soot samples (n=10 for diesel and biodiesel samples)*

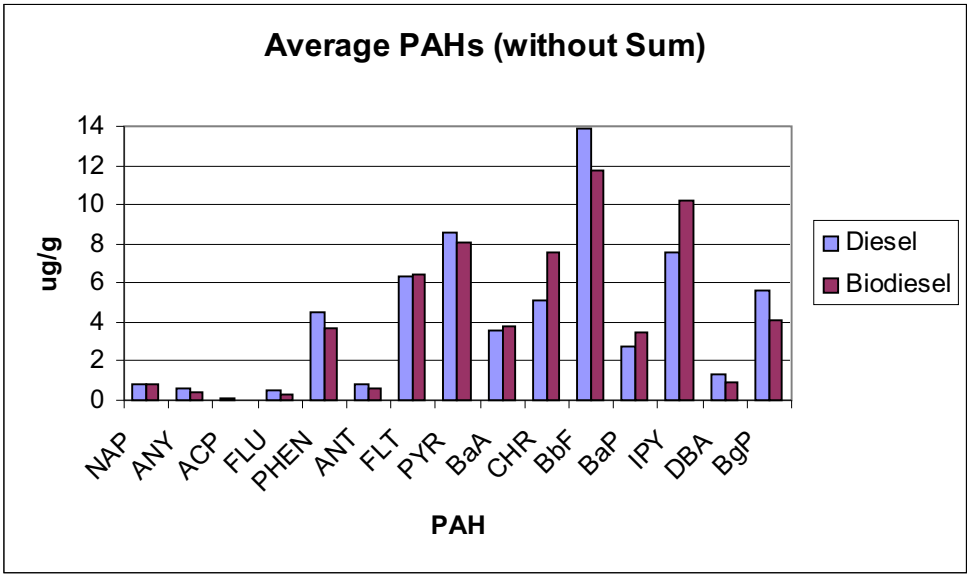

Figure 2B. *Comparison of average individual PAH levels in diesel and biodiesel exhaust soot (without Sum). Ten out of the fifteen analyzed PAHs were higher in diesel as compared to biodiesel samples.*

result (Fig. 2A), Figure 4A clearly showed higher total PAHs (or SUM) for the 2nd category (toxic all dilutions) compared to the first. Overall, the data generally showed that more of the individual PAHs as well as higher total PAHs occurred in the "toxic all dilutions" group compared to the "toxicity decreases with dilution" group.

Our results are in agreement with previous findings on the presence of known PAHs, such as pyrene, fluoranthene, phenanthrene, and higher molecular weight PAHs in the soot samples and their highly pronounced teratogenic effects on the zebrafish embryos [3,6,14,19,28]. The most commonly observed abnormalities in our test were the development of lordosis (spinal curvature), edema (pericardial and yolk-sac), cardiac and cranio-facial abnormalities [33] and that the observed developmental defects also exhibited dose-dependence. Regarding the mechanisms of embryotoxic responses, a number of studies have already elucidated the mechanistic effects due to individual PAH and PAH mixtures. Most PAHs, especially the heavier ones (>4 rings), are known to act as dioxin-like substances that can bind to the aryl hydrocarbon receptor (AhR) and induce cytochrome P450 monooxygenase [6,20]. These enzymes may lead to either detoxification of xenobiotics or conversion of PAHs into

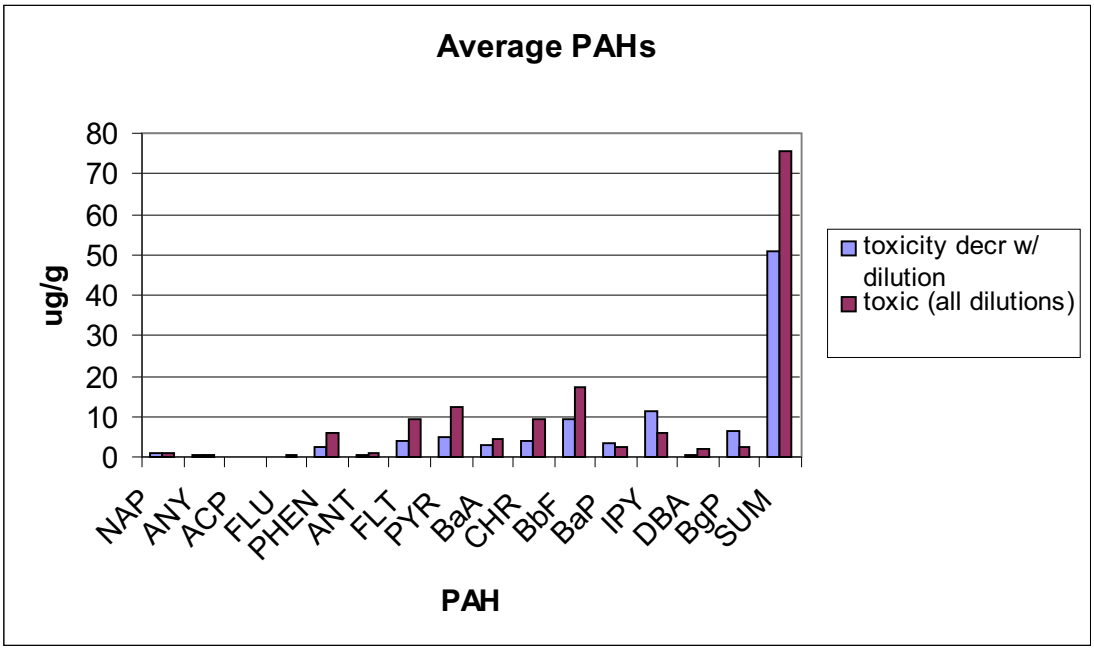

Figure 3A. *Comparison of PAH average levels in terms of toxicity using zebra fish assay (Note: diesel and biodiesel soot samples are present in each category)*

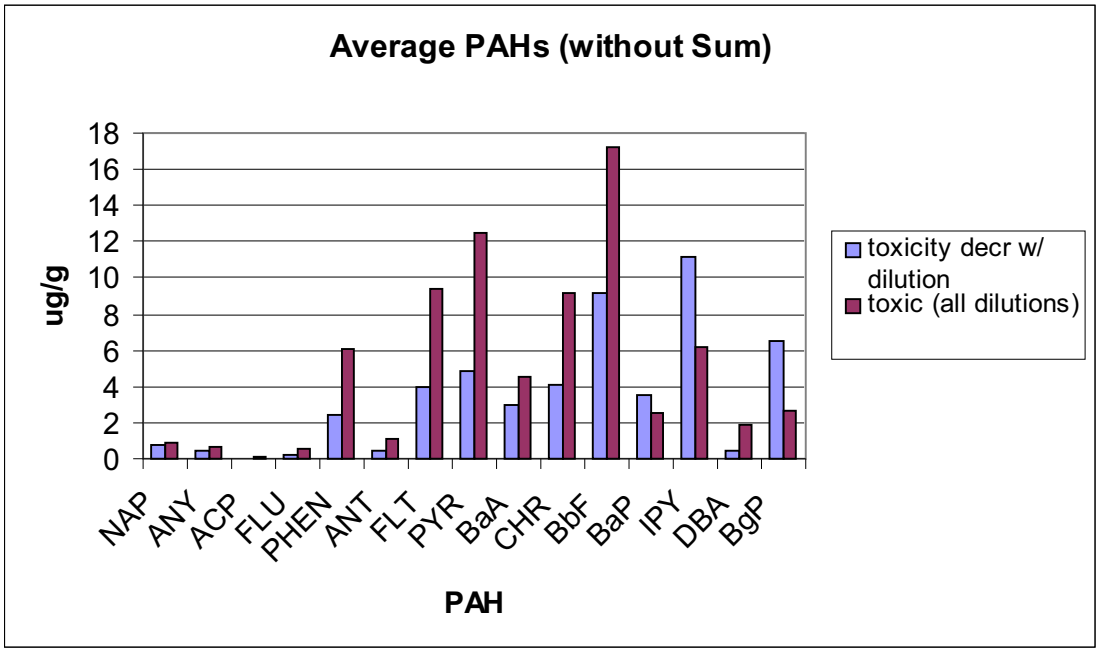

Figure 3B. *Comparison of PAH average levels (without Sum) in terms of toxicity using zebra fish assay. (Note: diesel and biodiesel soot samples are present in each category)*

more toxic metabolites eliciting a myriad of toxic effects including hepatotoxicity, embryotoxicity, teratogenicity, endocrine disruption, immunotoxicity, dermal toxicity, lethality, carcinogenesis, and wasting syndrome in many species at low concentrations [27,34,35]. There were studies, however, that reported no positive correlation observed between cytochrome P450 induction and mortality [6]. Meanwhile, one other mechanism of mortality is the exhaustion of energy stores in terms of liver glycogen depletion [36].

The heart was observed to be the most sensitive indicator of toxicity of PAH (i.e. pericardial edema) as reported elsewhere [17,28,37]. In a recent study by Zhang *et al.* [38], low-level pyrene-treated zebrafish embryos showed dose-dependent heart abnormalities, such as

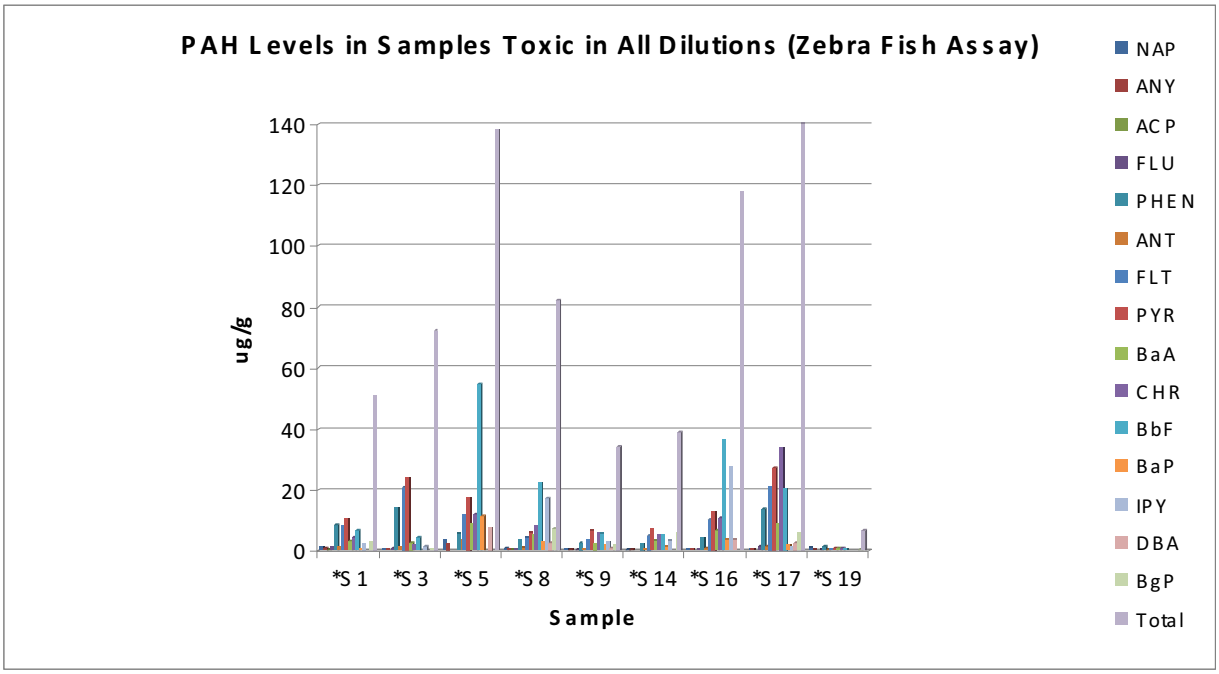

Figure 4A. *Summary of PAH levels in individual soot samples (100 % toxicity for all dilutions used in zebra fish assay). *S1, *S3, *S5, *S8 and *S9 are diesel soot samples while *S14, *S16, *S17 and *S19 are biodiesel soot samples.*

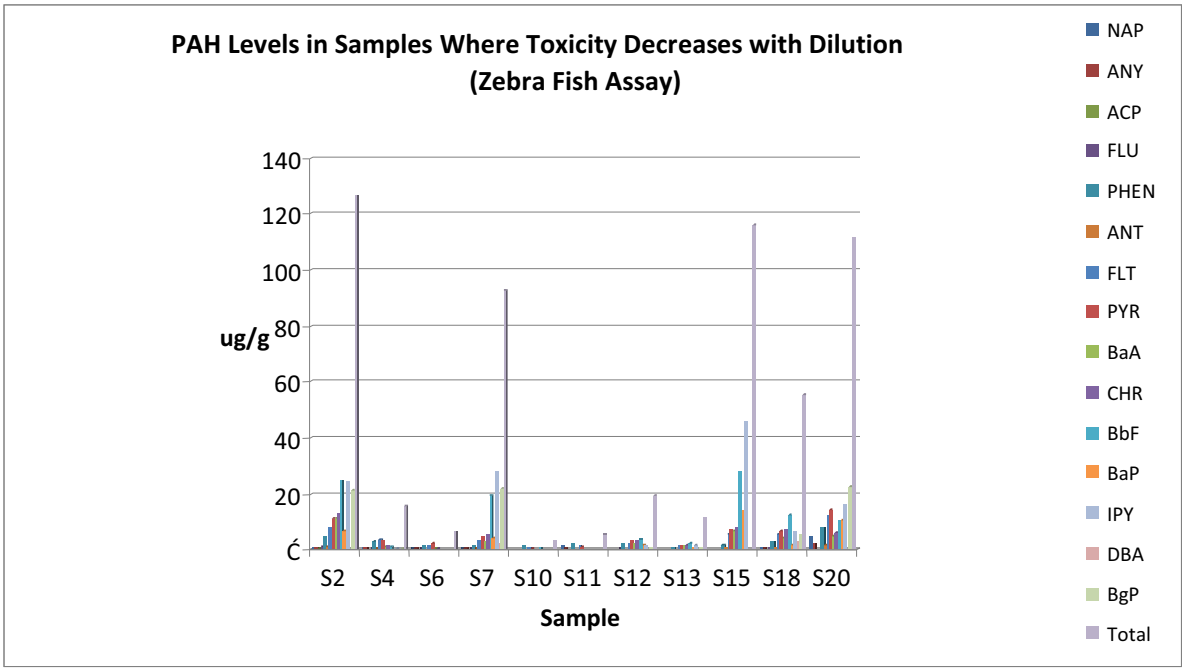

Figure 4B. *Summary of PAH levels in individual soot samples (decreasing toxicity with dilution used in zebra fish assay). S2, S4, S6, S7 and S10 are diesel soot samples while S11, S12, S13, S15, S18 and S20 are biodiesel soot samples.*

pericardial edema and cardiac looping defects and this was attributed to the down-regulation of the homeodomain transcription factor Nkx2.5, which is implicated in the development of the cardiovascular system. Leaks in endothelial cells [17] preceding the formation of pericardial edema may also result in specific cardio-vascular dysfunctions eg. changes in rate, rhythm, and contractility) as soon as the heart becomes functional [6]. Furthermore, these alterations in cardiac integrity in embryos can also be carried later in development as shown by a reduced aerobic capacity in adult [21].

Meanwhile, the developing embryos exposed to soot extracts also showed impaired skeleton development, such

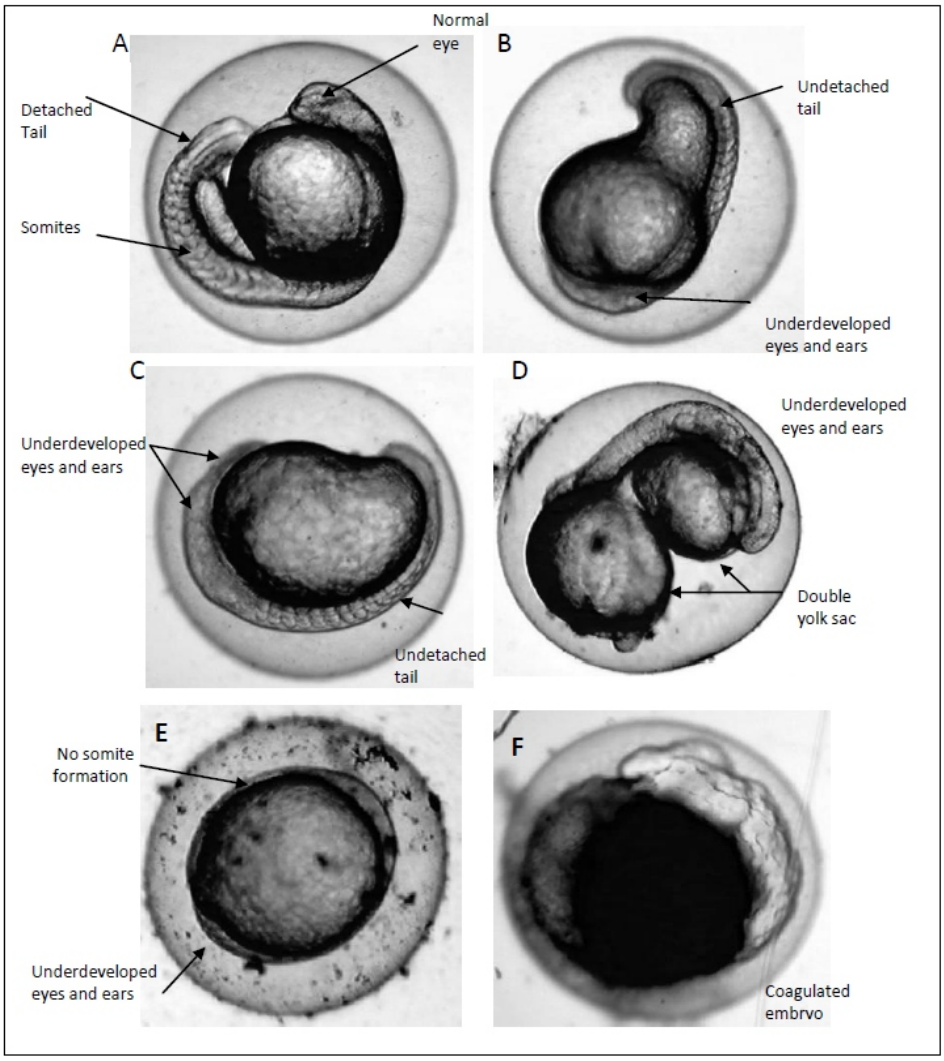

Figure 5A. *Normal 24-h embryo from reconstituted ISO water. B.-C. Underdeveloped embryo (tail not detached, underdeveloped eye and ear). D. Double yolk sac appeared in S7 and S11. E. Delayed embryo with no somites, tail undetached, and underdeveloped eye and ear from S6, and F. A coagulated 24-h embryo from S3.*

as craniofacial and spinal malformations. According to two recent studies, these aberrations may be due to the disruption of chondrocyte proliferations after exposure to pyrene [33] and benzo(a) pyrene [14]. Two more studies have implicated the spinal malformations with the depletion and deregulation of calcium and phosphorus ions or with a reduction in myosin proteins [39,40].

Carls *et al.* [28] reported that the dissolved forms of PAHs are responsible for known toxicities in fish embryos and that the mere physical contact with oil droplets is not necessary for embryotoxicity. In our experiment, we have exposed the eggs in highly dissolved PAHs present in the soot and that would account for almost 100% mortality in all test solutions irrespective of whether obtained from diesel or biodiesel sources.

In comparing the embryotoxic potential of petrodiesel and biodiesel soot extracts, it is very clear from our results that despite the relatively higher number of PAHs and higher total PAHs in petrodiesel, the toxicity is comparable to that of the biodiesel soot extracts. Both types of extracts are well represented in the 'toxic in all dilutions' as well as in 'toxicity decreases with dilution' groups. One plausible explanation is the low biodiesel content of the blend (only 1% biodiesel in diesel). The present study represents one of the first few attempts to compare the relative potencies of petrodiesel with biodiesel soot extracts in terms of embryotoxicity endpoints. Past studies including the most recent ones were mainly focused on comparing the mutagenicity of the two types of diesel [12,13,41,42]. In general, there is a substantially lower mutagenicity of biodiesel compared to the petrodiesel due to the lower

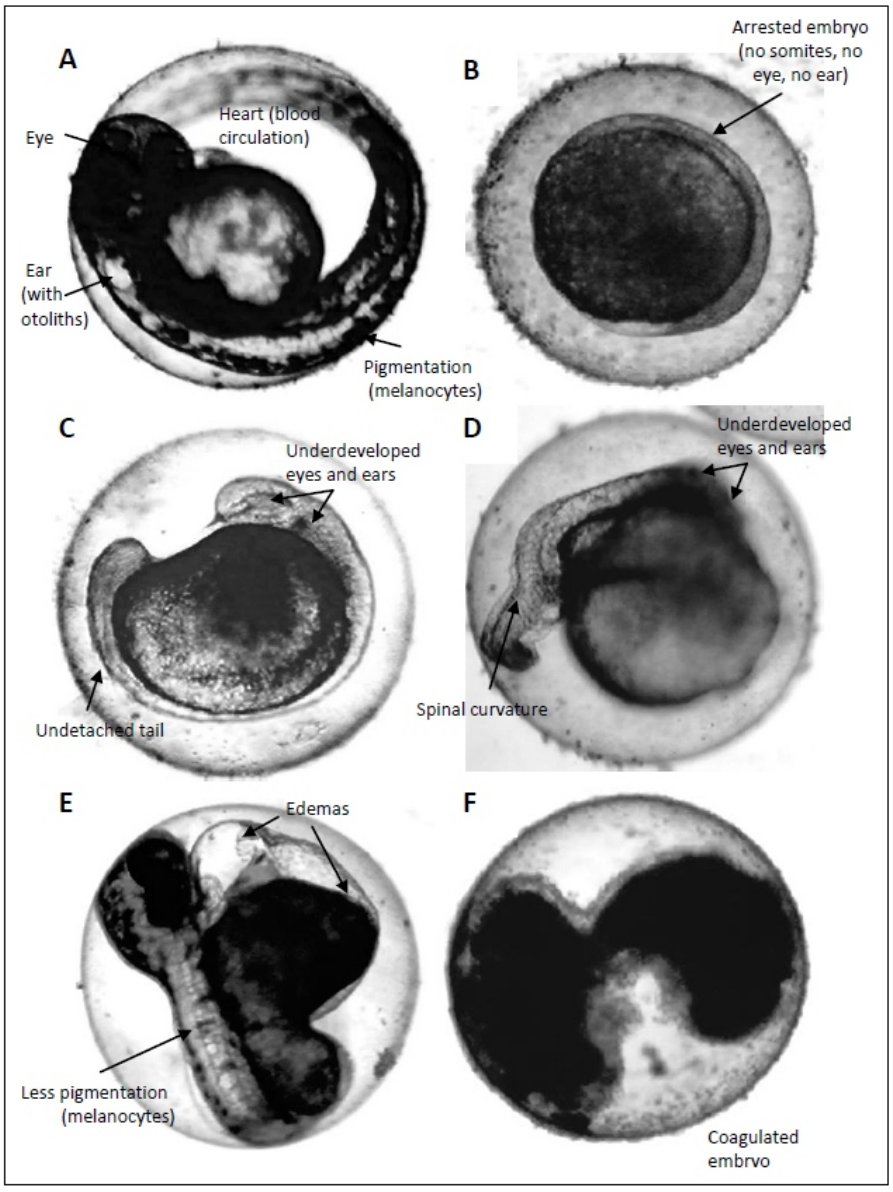

Figure 6A. *Normal 48-h embryo obtained from solvent control. B. An underdeveloped embryo that remained in late gastrula with no somite formation (from S8). C. An embryo showing underdeveloped head region, undetached tail, and less pigmentation (from S4). D. An embryo with deformed spine (from S2). E. An embryo showing fused yolk sac and edema (from S11 and S18). F. A coagulated embryo from positive control and also observed in most samples.*

content of polycyclic aromatic compounds, especially of pyrene, phenanthrene, 2-methylanthracene, 3-methylphenanthrene, and fluoranthene. In addition, biodiesel also contains very low amounts of sulphur and nPAHs. Very few studies have attempted to investigate cytotoxicity, apoptosis, and Ah-receptor affinity with biodiesel [43,44], and the results were either contrasting or inconclusive. For a review of limited studies on the use of these mechanism-specific toxicity assays, please refer to Bluhm *et al.* [45].

Through the use of another assay (i.e. zebrafish embryo assay), the present study supported the presence of embryotoxicity inducers in both petrodiesel and biodiesel types. With the continuing hype and emerging interest on the use of biofuel to replace the conventional fossil fuels, there is a need to carry out further investigations for its environmental costs and benefits. While it is highly accepted that the use of biofuels promises reduction of greenhouse gas emissions and slowing down of the impact of global climate change, equal considerations must be

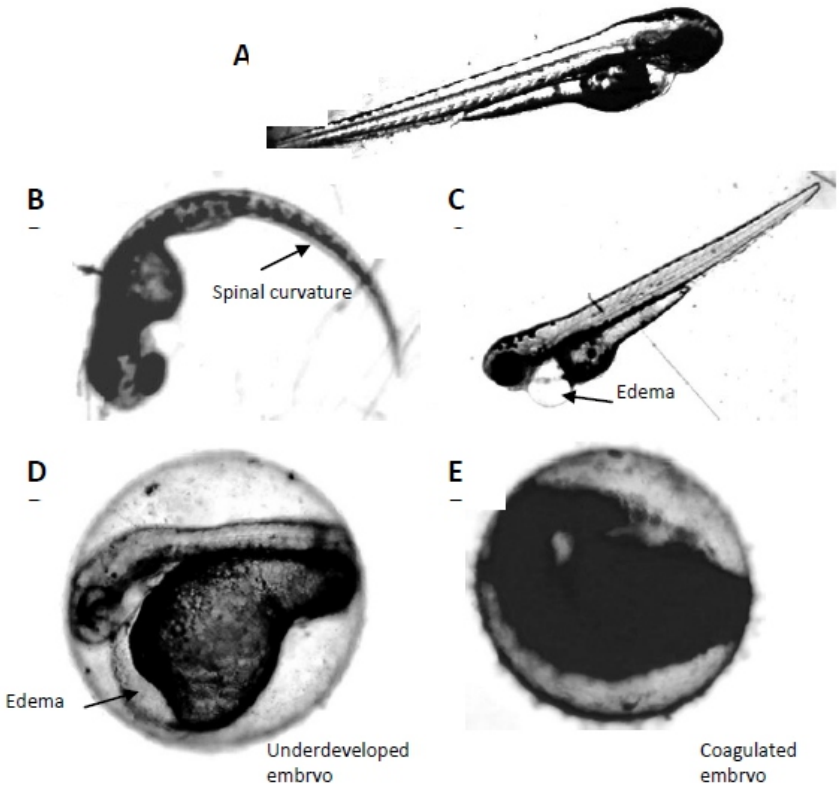

Figure 7A. *Normal 72-h embryo obtained from ISO control with well-developed organs and normal pigmentation. B. An embryo with spinal curvature (lordosis) from S12. C. An embryo with cardiac edema from S6. D. An embryo that is underdeveloped, unhatched, and cardiac edema from S2. E. A coagulated 72-h embryo.*

given to the possible hazards it can pose to human and ecosystem health. We recommend further research on the ecotoxicological potencies of biofuels to fill in data gaps and to unify existing discrepancies on toxicity test results before we can fully accept biodiesel as the most suitable alternative to conventional fossil fuels.

Conclusions

The present study attempted to assess the levels as well as the ecotoxicological hazards of PAH-rich soot extracts collected from the tailpipes of public utility jeepneys plying along a metropolitan city. Seven PAHs were consistently found in both the diesel and biodiesel extracts: phenanthrene, pyrene, fluoranthene, chrysene, benzofluoranthene, indeno pyrene, and benzoperylene. PAHs were generally present and of comparable levels in both extracts. Nevertheless, in terms of average levels of particular PAHs, the diesel extracts contained more types of PAHs that registered higher levels than those of biodiesel (10 against 4). All undiluted samples from all extracts were embryotoxic to zebrafish embryos wherein they coagulated in a matter of 12-24 hours. Diluted samples in some extracts have shown decreasing level of toxicities as the dilution levels increased. However, a number of abnormalities and lethal endpoints have been observed due to exposure to PAH-rich soot extracts. The results described here could aid in a development of a rapid-response model for predicting teratogenic potential of combustion-derived soots in a broad range of

vertebrates. More researches are necessary to determine the actual concentrations in different locations and environmental matrices to further assess the actual environmental and human health risks of these priority pollutants.

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