



Combined toxicity effects of MTBE and pesticides measured with *Vibrio fischeri* and *Daphnia magna* bioassays

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Abstract

Methyl-tert-butyl ether (MTBE), a fuel oxygenate that is added to gasoline, commonly contaminates aquatic systems, many of which are already contaminated with pesticides. The toxic effects (EC₅₀ value) of several pure pesticides (Diuron, Linuron, Dichlofluanid, Sea nine, Irgarol and tributyltin (TBT)) were measured and compared with the EC₅₀ value of the pesticide mixed with MTBE, using the *Vibrio fischeri* and *Daphnia magna* acute toxicity assays. The interaction between chemicals was evaluated in terms of the effects of mixing on the EC₅₀ value (i.e. the concentration (mg/L) of a compound or mixture that is required to produce a 50% change in a toxic response parameter) and the time required to generate the toxic response. Presence of MTBE enhanced the EC₅₀ value of several pesticides (Diuron, Dichlofluanid, TBT and Linuron) and/or the toxic response manifested more rapidly than with pure pesticides. Toxicity enhancements were quite substantial in many cases. For example, the presence of MTBE increased the toxicity of Diuron by more than 50% when tested with the *V. fischeri* assay (5, 15 and 30 min exposure). Also, the toxic response manifested itself within 5 min whereas without the MTBE the same response arose in 30 min. Presence of MTBE increased the toxicity of Dichlofluanid by 30% when measured with the *D. magna* assay. Toxicities of only two pesticides (Sea nine and Irgarol) were not raised by the presence of MTBE.

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1. Introduction

Because of its widespread use as a fuel oxygenate in gasoline, methyl-tert-butyl ether (MTBE) commonly contaminates groundwater, drinking water [1], coastal waters, and other aquatic bodies. MTBE concentrations as high as 200 mg/L have been detected in groundwater supplies located close to leaking underground gasoline storage tanks. MTBE concentrations of 0.088 mg/L have been found in lakes [1] and concentrations in coastal waters can range from 0.005 to 0.8 mg/L [2].

In response to the inputs of MTBE into aquatic ecosystems, numerous toxicity studies on different marine species and freshwater organisms have been reported. Acute and chronic toxicity of MTBE alone appears to be low towards marine species [3]. However, in the environment, species are exposed to large number of chemicals, and therefore, the toxicological studies about the adverse effects and potential risk of the mixtures of chemicals, is growing. How the combination of MTBE and pesticides affects the toxicities of individual compounds is an important question that impacts on the health of the aquatic environment. This work evaluates the effects of MTBE on toxicity of the pesticides Diuron, Dichlofluanid, Sea nine 211, Irgarol 1051, Linuron and Tributyltin (TBT). These specific compounds are found frequently in marinas, rivers, and lakes [4–6]. Diuron, Linuron and Irgarol are biocides,

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which act as inhibitors of photosystem-II and are used to eradicate algae. Dichlofluanid is widely used as a protective fungicide in agriculture. Sea nine is broadly toxic to marine bacteria, algae and barnacles [7]. TBT, an active ingredient of antifoulant paints used on ships, is extremely toxic to aquatic organisms [7].

Compounds in mixtures are known to interact with biological systems in ways that can greatly alter the toxicity of individual compounds [8–11]. Mixture effects on the organisms can be antagonistic, additive or synergistic. For the effect assessment of mixtures, three theoretical concepts of joint action (“Simple similar action” and “Simple dissimilar action”) or interaction of combination of chemicals have been widely used in toxicological studies [12]. The models of joint action allow us to describe mathematically, the additive effects—considering that the chemicals of a mixture act by non-interactive processes. However, when the combined effect resulting in a stronger (synergism, potentiation) or weaker effect (antagonism, inhibition) than that expected on the basis of additivity, interactive processes are considered. For these cases, the mechanisms of physicochemical and/or biological nature are considered as well as the interaction which can occur in the toxicokinetic phase (processes of uptake, distribution, metabolism and excretion) or/and toxicodynamic phase (effects of the chemicals on the receptor, cellular target or organ). For example, induction and inhibition of an enzyme by one toxicant can modify an organism’s response towards other toxicants. Also, one compound may compete with another for participation in a metabolic process [12].

For the assessment of the contamination in aquatic ecosystems, many types of bioassays have been used. The test organisms incorporated in these bioassays include species of different group as microorganisms, plants, invertebrates and fish [13]. Zooplankton (in particular the genus *Daphnia*) is frequently used in ecotoxicological tests because this is one of the groups most sensitive to toxic chemicals and this organism occupies a central position in the lentic food chain [14]. Bacteria *Vibrio fischeri* is another test organism widely accepted in toxicity test. Both *V. fischeri* and *D. magna* assays are rapid, relatively inexpensive, simple and reproducible [15–18]).

The goals of this study were

- To evaluate the individual and combined toxic effects of different pesticides and the fuel oxygenate (MTBE) using bacteria (*V. fischeri*) and crustacean (*Daphnia magna*). The toxic effects were quantified as EC_{50} value (i.e. the concentration (mg/L) of a compound or mixture that is required to produce a 50% change in a toxic response parameter).
- To evaluate the time required to generate the toxic response on *V. fischeri* and *D. magna* assays.

2. Material and methods

2.1. Test chemicals

The following contaminants were tested individually and in combination with MTBE: Irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine), Sea nine 211 (4,5-dichloro-2-(n-octyl)-3(2H)-isothiazolone), Dichlofluanid (N-dimethyl-N-phenylsulfamide), Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) and TBT. All chemicals used were purchased in the highest purity available from Ciba-Geigy (Barcelona, Spain), Rhom & Hass (Philadelphia, USA), Chemservice (West Chester, USA) and Riedel-de Haën (Seelze, Germany).

Stock solutions of mixtures, individual pesticides and MTBE were prepared in distilled water for the *V. fischeri* assay and in the culture medium for the *D. magna* bioassay. For *V. fischeri* assay, the osmolality of solutions was adjusted to 2% aqueous sodium chloride for optimal performance of the assay. When a compound had a low solubility in water, 0.2% dimethyl sulfoxide was used to dissolve it. Dimethyl sulfoxide (DMSO, 97%) was purchased from Merck (Dusseldorf, Germany). Control experiments carried out with equivalent volumes of DMSO exhibited no observable toxic effect on the assay species.

Mixtures were prepared to contain MTBE at a concentration of 0.1 mg/L and the individual pesticides at their EC_{50} effective concentration levels. The concentration level for MTBE was selected according to concentration levels which have been found in the aquatic environment such as lakes [1] or in coastal waters which range from 0.005 to 0.8 mg/L [2].

EC_{50} values for *V. fischeri* and *D. magna* of the pesticides and MTBE are documented in Table 1. Only the mixture of MTBE and TBT was prepared at a TBT concentration level that was less than the EC_{50} of pure TBT because of its extreme toxicity and in this way, to obtain more information about the development of the toxic response with respect to time. The TBT concentration in the mixture was 0.8 ng/L.

2.2. *Vibrio fischeri* bioluminescence assay

The effect of test compounds on the luminescent bacterium *V. fischeri* was evaluated using the Biotox™ test with 5, 15 and 30 min exposure (Bio-Orbit Oy, Turku, Finland). The bacteria were purchased as freeze-dried reagents and stored at -20°C . The cells were rehydrated prior to use. The metabolic pathway responsible for light emission by the bacterium is intrinsically linked to cellular respiration and any disruption of normal cellular metabolism causes a decrease in light production. The toxicity end point (EC_{50}) was determined as the concentration of a test

Table 1
EC₅₀ values for single pesticides analyzed by *V. fischeri* and *D. magna* toxicity assays

| Pesticide | EC ₅₀ (mg/L) | |
|---------------|---------------------------------------|--|
| | <i>V. fischeri</i> 30 min exposure | <i>D. magna</i> 48 h exposure |
| Linuron | 5.5 ± 1.1 | 7 ± 1.2 ^a |
| Sea nine | 0.003 ± 0.0003 | 0.004 ± 0.0007 |
| Irgarol 1051 | 15.5 ± 2.7 | 7.3 ± 1.24 |
| Diuron | 100 ± 7.8 | 8.6 ± 1.3 |
| Dichlofluanid | 0.06 ± 0.0118 | 1.05 ± 1.33 |
| TBT | 0.007 ± 0.0012 | 1 × 10 ⁻⁶ ± 1.2 × 10 ⁻¹⁰ |

Data are expressed as mean ± standard deviation ($n = 6$ replicates).

^a EC₅₀ value of Linuron on *D. magna* was for 96 h exposure.

sample that caused a 50% reduction in the light output. The acute bioluminescence assay was carried out according to ISO 11348 [19]. Light emission of the bacterial suspension was measured with a photomultiplier in a luminometer equipped with a constant temperature water bath (15°C).

2.3. *Daphnia magna* motility assay

The toxicity of compounds for the crustacean *D. magna* (Cladocera) was assessed using a commercially available kit (Daphtoxkit™; Creasel, Belgium). The toxicity studies were performed in accordance with testing conditions prescribed by OECD Guideline 202 and ISO 6341 [20]. Culture medium was prepared mixing non-chlorinated tap water and spring water 1:1, to obtain 170 mg/L CaCO₃.

Acute toxicity was assessed by noting the effects of the test compounds on the motility of *D. magna*. The bioassay used 24-h old daphnids hatched from the dormant eggs (ephippia) according to the prescribed standard procedure for inducing the hatching. The tests were conducted in the dark at a constant temperature of 20 ± 1°C. The neonates were considered immobile if, after 24 and 96 h of incubation with the toxicant, they remained settled at the bottom of the multiwell test plate and did not resume swimming within 15 s of observation. The toxicity end point (EC₅₀) was determined as the concentration required to immobilize 50% of the daphnids after 24 and 96 h exposure.

2.4. Data analysis

2.4.1. Toxicity indices

For a more convenient interpretation of toxicity data, the results obtained as 50% effect point values were converted into a Mixture Toxicity Index (MTI) for comparing quantitatively the results of the mixture

Table 2
Mixture toxicity scale after Könemann

| MTI | Classification of toxicity mixtures for possible type of joint action |
|-------------|---|
| MTI < 0 | Antagonism |
| MTI = 0 | No addition (independent action, $r = +1$) ^a |
| 0 < MTI < 1 | Partial addition |
| MTI = 1 | Concentration addition (simple similar action) |
| MTI > 1 | Supra-addition (potentiation of the toxic action(s) of one or more of the compounds of mixture) |

^a Positive correlation between susceptibilities of the individual organism to the single toxicants.

toxicity, with the formula of Könemann: $MTI = 1 - \log M / \log n$, where $M = \sum TU_i$. This index was proposed on the basis that the chemicals in mixture act with simple similar action and independent action. MTI is calculated by using toxic units (TU) which are defined with the formula of Sprague and Ramsay [21]: $TU = [1/EC_{50}]$ and considering the number of compounds in the mixture (n). Effects of the mixture can be evaluated by this index in a toxicity scale proposed by Könemann [30] (Table 2).

2.4.2. Variability

The reproducibility of the *V. fischeri* and *D. magna* response were evaluated by replicating EC₅₀ tests six times. For this purpose, the EC₅₀ measurements of each compound or mixture for each assay were performed using the same toxicant solution, and the same batches of the freeze-dried bacteria or the dormant eggs of *D. magna*. Each toxicant solution was analyzed each day for 6 days.

The average reproducibility obtained as medium value of the EC₅₀ measurements was ±24% and ±26% for the *V. fischeri* and *D. magna* systems, respectively (Table 1). These reproducibility values were comparable to 10–20% reproducibility previously reported [15,16] for the *V. fischeri* assay. Reproducibility from several intra- and inter-laboratory studies reported an average reproducibility of 38.8% for the bioassays based on the most common invertebrate and fish test species [22]. Our values are consistent with these norms.

2.5. Chemical analyses: study of the disappearance of MTBE by volatilization

MTBE is highly water soluble (40–51 g/L solubility at 20–25°C), highly volatile and a persistent pollutant. Because the toxicity assays ran over a considerable length of time, the possible disappearance of MTBE by

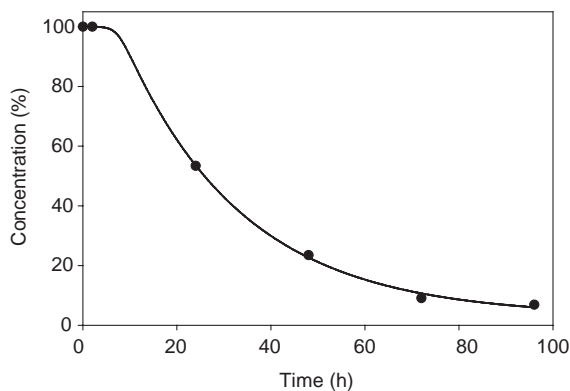


Fig. 1. Volatilization rate of MTBE in the experimental conditions at 22°C.

volatilization under the assay conditions had to be assessed. Thus, aqueous solutions of MTBE (1 mg/L initial concentration) were held at 22°C for various lengths of time and analyzed for MTBE by GC-AED (atomic emission detection) in intervals of time ranging from 0 to 100 h. The observed time course of decline in MTBE concentration is shown in Fig. 1. Up to about 50% of the initial MTBE was lost by volatilization in 24 h (Fig. 1).

Despite its high rate of volatilization, the MTBE loss by evaporation was not relevant to the *V. fischeri* assay which lasted for 30 min at most. During this period, only an insignificant amount of MTBE volatilized, as shown in Fig. 1. In contrast, the *D. magna* toxicity assay ran for up to 96 h and was potentially susceptible to MTBE loss by volatilization. In view of this, the toxicity bioassays were conducted in a flow-through mode as well as in the standard static mode. In static experimental conditions, the test organisms were exposed to the toxicant at the required concentration by replacing a batch of toxicant with a fresh batch of the same initial concentrations in frequent intervals of time (5 h), and in flow-through tests, the toxicant was introduced at regular intervals with a corresponding outflow (24 h). The toxicity responses for the two modes of testing were quite similar for any given solution, suggesting an absence of MTBE loss by volatilization in both systems.

3. Results and discussion

3.1. Toxicity of pure MTBE

Reported data on toxicity of MTBE suggest that this compound has a low toxicity towards aquatic organisms [3,1], as can be seen in Table 3. Depending on the length

Table 3
Toxic effects (EC₅₀) of MTBE in freshwater organisms

| Species | Measured effect/ concentration (mg/L) | Reference |
|-------------------------|--|-----------|
| Invertebrates | | |
| <i>N. spinipes</i> | EC ₅₀ (96 h) > 1000 | [23] |
| <i>D. magna</i> | EC ₅₀ (96 h) = 681 | [24] |
| | EC ₂₅ (96 h) = 57 | [25] |
| <i>M. bahia</i> | EC ₅₀ (96 h) = 136 | [24] |
| Vertebrate | | |
| <i>O. latipes</i> | LOEC ^a (8 days) = 2600 | [27] |
| <i>O. mykiss</i> | EC ₅₀ (96 h) = 1237 | [24] |
| <i>P. promelas</i> | LOEC ^a (7 days) = 388 | [26] |
| Bacteria | | |
| <i>S. typhimurium</i> | EC ₅₀ (48 h) = 7.4 | [13] |
| <i>P. phosphoreum</i> | EC ₅₀ (15 min) = 41.8 | [22] |
| Algae | | |
| <i>S. capricornutum</i> | EC ₅₀ (72 h) = 184 | [28] |
| | EC ₅₀ (5 days) = 4800 | [24] |
| <i>S. leopoliensis</i> | EC ₅₀ (3 days) = 2400 | [28] |

^aLOEC = lowest observable effect concentration.

of exposure, MTBE is toxic to invertebrates at concentrations of 57 to >1000 mg/L [23–25]. At concentrations of 388–2600 mg/L, MTBE is toxic to vertebrates [24,26,27]. EC₅₀ values of 7.4–41.8 mg/L have been reported for acute toxicity of MTBE towards bacteria such as *Salmonella typhimurium* and *Photobacterium phosphoreum* [22,13]. In microalgae, MTBE is toxic at concentrations of 184–4800 mg/L when tested with species such as *Selenastrum capricornutum* and *Synechococcus leopoliensis* [24,28]. Our data suggest an EC₅₀ value of 12 mg/L for toxicity of MTBE for *V. fischeri* at 30 min of exposure. Clearly, *V. fischeri* is more sensitive than *P. phosphoreum* even though the toxicity response of both bacteria is measured as a reduction in bioluminescence [13,22].

Our value of MTBE's toxicity on *D. magna* (EC₅₀ = 720 mg/L) is consistent with a reported 96-h EC₅₀ measurement of 681 mg/L [24]. Other authors have claimed that MTBE is not toxic to *D. magna* at concentrations of 1–1000 mg/L for exposures up to 48 h [22]. These results, obtained with the static mode of the *D. magna* assay, are explicable by volatilization of MTBE. Volatilization can greatly reduce the concentration of MTBE seen by the test organism over the duration of the assay.

Considering our EC₅₀ values of 12 (*V. fischeri*) and 720 mg/L (*D. magna*), the typically found environmental levels (0.088–0.200 mg/L) of MTBE are unlikely to harm

aquatic species so long as bioaccumulation is not a factor and MTBE does not interact with other pollutants to produce a toxic response [1].

3.2. Toxicity of MTBE in combination with pesticides

A discussion of toxicity of MTBE mixed with other compounds requires a knowledge of toxicities of the pure components of the mixture. The toxicity of pure MTBE has already been noted in an earlier section of this work. Toxicity values (EC_{50}) for the pure pesticides are summarized in Table 1 for the two test organisms.

Using the *V. fischeri* assay, the pure pesticide toxicity had the following order: Sea nine 211 > TBT > Dichlofluanid > Linuron > Irgarol > Diuron. A similar pattern was observed with the *D. magna* assay: TBT > Sea nine > Dichlofluanid > Linuron \geq Irgarol > Diuron. For three compounds (Irgarol, Diuron and TBT), the *D. magna* assay was much more sensitive than the *V. fischeri* test system. Both the test systems were similarly sensitive to Linuron and Sea nine. TBT and Sea nine were the most toxic compounds (Table 1) with both the assays. With both assay systems, the toxicities of all pesticides were substantially greater than pure MTBE toxicity (see previous section).

With both assay systems, joint effects evaluated with the MTI showed values which ranged from -5 to 6.9 for *V. fischeri* and from -4.6 to 6.3 for *D. magna* (Table 4). According to this theoretical approach, 66% of the mixtures will show a stronger effect than expected, on the basis of the additive model (synergistic or potentiation) for both bioassays. About 25% of mixtures show an antagonistic effect and 12% of the samples show a partial addition. None of the tested mixtures showed a simple additive response.

Combinations of MTBE and single pesticides were significantly more toxic than the respective pure pesticide in several cases. Also, with several mixtures, there was an accelerated development of the toxic response, compared to the pure pesticides. For all tested mixtures analyzed, only toxicity data obtained in both aspects are shown. As shown in Fig. 2, presence of

MTBE caused a 50% greater enhancement in toxic response relative to pure Diuron. Furthermore, a mixture of MTBE with Diuron caused a 50% response (EC_{50}) in 5 min while 30 min was necessary to cause the same effects for the single pesticide. The results in Fig. 2 are for the *V. fischeri* assay at exposure values of 5, 15, and 30 min. For the *D. magna* assay, no change in the toxic response of the mixture was observed. The different response in terms of time required to generate the toxicity, obtained in *V. fischeri*, could be explained on the basis that the interaction between both compounds might occur in the toxicokinetic or toxicodynamic phases. Active processes of uptake through the cellular membrane or active processes of binding to the receptor might be responsible for the generation of a toxic effect in a shorter time than the toxic effect of an individual pesticide.

As shown in Fig. 3, the toxic response (i.e. the EC_{50}) of *D. magna* for the MTBE-Dichlofluanid mixture developed more rapidly with the duration of exposure than the toxicity response for pure Dichlofluanid. Furthermore, the toxic response obtained in the mixture was higher than the individual pesticide. For 72-h exposure, both the pure pesticide and the mixture made all daphnids immobile (i.e. a 100% response in Fig. 3). However, for *V. fischeri*, the toxic response in both cases was the same.

Toxicity responses for pure TBT and TBT mixed with MTBE are compared in Fig. 4. TBT is known to bioaccumulate and is chronically and acutely toxic [7]. The EC_{50} values obtained in this work indicate its extreme acute toxicity (EC_{50} value of $7 \mu\text{g/L}$ (*V. fischeri*) and 1 ng/L (*D. magna*) in Table 1). Therefore, as it was

Table 4
Theoretical approach of toxicity of mixtures using MTI^a

| Mixtures | <i>V. fischeri</i> | <i>D. magna</i> |
|--------------------|--------------------|-----------------|
| Diuron–MTBE | 6.7 | 6.3 |
| Dichlofluanid–MTBE | -0.7 | 3.4 |
| TBT–MTBE | 6.9 | 0.3 |
| Linuron–MTBE | 5.2 | 6.1 |
| Irgarol–MTBE | 6.1 | 6 |
| Sea nine–MTBE | -5 | -4.6 |

^a MTI determined using experimental data (EC_{50}) of individual compounds.

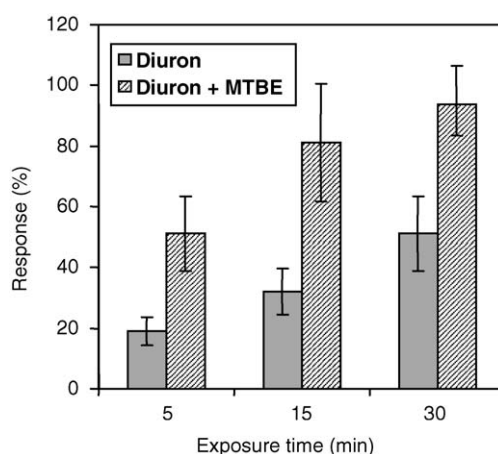


Fig. 2. Toxicity (*V. fischeri* assay; 5, 15 and 30 min exposure) response enhancement achieved by adding MTBE to Diuron. Vertical lines indicate \pm one standard deviation. All mixture effects are significantly different at the 5% level with respect to the single compound effect, by applying significance test.

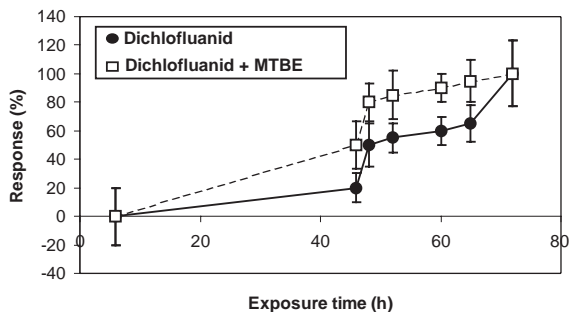


Fig. 3. Toxicity (42h exposure, *D. magna* assay) response enhancement caused by combining MTBE with Dichlofluanid. Vertical lines indicate \pm one standard deviation. The mixture effects calculated at 45, 48 and 60h of exposition are significantly different at the 5% level with respect to the single compound effect, by applying significance test.

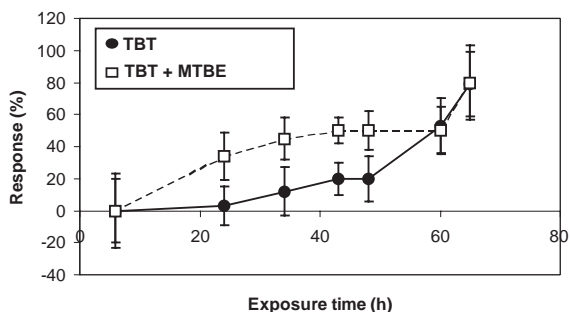


Fig. 4. Toxicity (42h exposure, *D. magna* assay) response enhancement caused by combining MTBE with TBT. Vertical lines indicate \pm one standard deviation. The mixture effects calculated from 24–48 h of exposition are significantly different at the 5% level with respect to the single compound effect, by applying significance test.

noted in the experimental section, the mixtures of TBT and MTBE were formulated to contain TBT at a sublethal concentration (0.8 ng/L), or a lower concentration than the EC₅₀ value of pure TBT. For up to 48 h of exposure, the toxicity response for the mixture developed more rapidly (*D. magna* assay) than for the pure TBT. And after 65 h of exposure, very similar responses were observed in both cases (Fig. 4). On *V. fischeri*, the behavior of the mixture and the TBT was the same.

Toxicity of TBT and MTBE mixtures is particularly relevant to coastal waters because TBT is frequently found in high concentrations especially in enclosed marinas (1.2–78 ng/L) [29].

Toxicity of Sea nine and Irgarol were unaffected by admixture with MTBE. As an example of both cases, in Fig. 5, mixing of MTBE with Irgarol did not affect the toxic response (*V. fischeri*) for any length of exposure

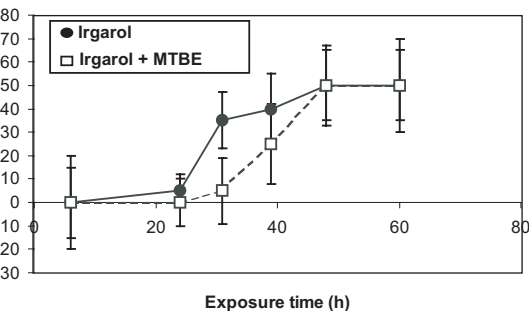
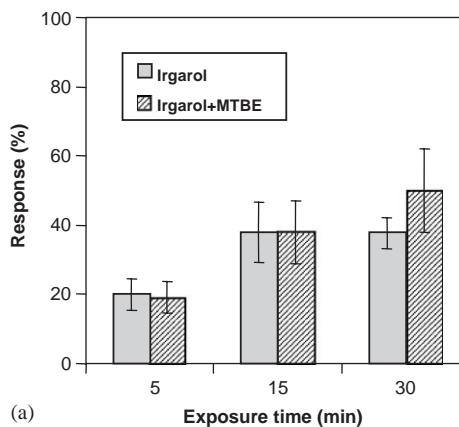


Fig. 5. Mixtures of Irgarol and MTBE produce a similar toxic response as does pure Irgarol: (a) analysis with the *V. fischeri* assay. The mixture effects calculated at 28 and 39 h of exposition are significantly different at the 5% level with respect to the single compound effect, by applying significance test, (b) measurements with the *D. magna* assay. Any mixture effect is significant at the 5% level with respect to the single compound effect, by applying significance test. Vertical lines indicate \pm one standard deviation.

(Fig. 5a). With the *D. magna* assay (Fig. 5b) and up to 48 h exposure, the toxic response developed a little faster for pure Irgarol than for the mixture. Concentrations of Irgarol in marinas range from 30 to 1000 ng/L [30]. These values are significantly less than the EC₅₀ of Irgarol measured with the two bioassays.

Toxicities (i.e. the EC₅₀ values) of mixtures of MTBE and Linuron were identical to that of pure Linuron when tested with the *V. fischeri* assay. With the *D. magna* assay, the toxicities of MTBE–Linuron and pure Linuron were broadly similar for less than 60 h of exposure. For 68 h exposure, Linuron was 15% more toxic than the mixture. Of all compounds tested, Linuron alone required an extended exposure (96 h) to produce a toxic response towards *D. magna*. The toxic response for MTBE–Linuron mixture developed approximately 2h earlier than the response for pure Linuron (Fig. 6).

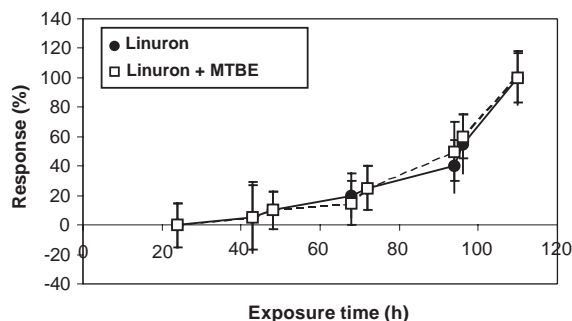


Fig. 6. Similar toxic responses of Linuron and mixture of Linuron with MTBE. Vertical lines indicate \pm one standard deviation. Any mixture effect is significantly different at the 5% level with respect to the single compound effect, by applying significance test.

4. Conclusions

MTBE alone is toxic to the tested species ($EC_{50} \geq 12$ mg/L) at concentrations that are significantly greater than the levels of this pollutant that are commonly detected in the environment. However, presence of MTBE can substantially enhance the toxicity of other pollutants such as pesticides which are often present in the same environment as MTBE.

Combinations of MTBE at 0.1 mg/L with EC_{50} or lower concentrations of pure Diuron, Dichlofluanid, TBT, and Linuron, are more toxic than the pure pesticides. Also, with combinations of MTBE and pesticides the toxic response is manifested more rapidly than with pure pesticides. Only for Sea nine and Irgarol, the addition of MTBE did not increase the toxicity relative to toxicities of pure Sea nine and Irgarol. For several mixtures (e.g. MTBE and TBT), toxicity was detected at concentrations lower than the concentration values commonly detected in the environment.

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