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Validation of SOS-*lux* Microbial Biosensors for Mutagenicity Assessment: Mitomycin-C as a Model Compound

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Abstract

Human health protection requires relating the bioaccessible concentration of a mutagen with the corresponding likely harm that could be caused through exposure. This requires designating the target receptor in need of protection, and a quantitative understanding of the likely pathways for mutagen availability. In this study, young children were selected as target receptors because of their tendency to directly ingest soils. Most data used to characterise a chemical mutagenicity has been extrapolated from rat-based assays using chemical ingestion or direct injection procedures. Mitomycin C was selected as a relevant model compound and extracted using an established *in vitro* digestion technique. A range of mutagenic bioassays (i.e. SOS-*lux* based microbial biosensors and *Salmonella* mutagenicity assay) were calibrated and optimised in aqueous samples, before being applied to soil extracts. The biosensors were consistently as sensitive and responsive as the traditional *Salmonella* assay, however, the use of microbial biosensors offered speed and ease of analysis. The data presented confirm that the *in vitro* digestion bioassay enabled a rapid and inexpensive technique for deriving critical values for the protection of humans exposed to soil borne mutagenic pollutants.

Keywords: SOS-*lux* biosensors; *Salmonella* assay; *In vitro* digestion; Mutagenicity; Bioluminescent bacteria; Mitomycin C

Introduction

Soil ingestion (both directly and through vegetable consumption) is a significant pathway by which pollutants enter the human body [1]. Permissible concentrations of ingested soil pollutants have been derived from animal studies (direct feeding or injection), and translated to humans. Such extrapolations often fail to consider the bioaccessible and bioavailable fractions of pollutants and their relative toxicity [2]. These extractions quantify the bioaccessible fraction of soil pollutants by simulating the oral exposure pathway for children (the most sensitive human receptors). The term, bioaccessible, in the context of human ingestion, is defined as the fraction of a substance that is soluble in the gastrointestinal environment and available to human biochemical processing [3].

Environmental mutagens are external agents that when activated, increase the rate of mutation in cells. Chemical analysis alone is unable to predict the biological impacts, antagonistic or synergistic effects in a mixture of mutagenic pollutants, nor to address the significance of bioavailability in terms of pollutant assimilation [4]. These shortcomings have required the development and application of rapid assays to screen large numbers of samples from environmental matrices.

Luminescence-based bacterial biosensors have been developed to detect a variety of mutagens [5]. Biosensors for mutagenicity assessments have been used due to their simplicity and sensitivity [6]. The SOS-*lux* based microbial biosensors have a promoter less *lux*-operon (*luxCDABE*) under control of the SOS-dependant *col* promoter, and thus, its synthesis is regulated by the SOS-system [7]. An exposure to a target analyte leads to an increase in the concentration of luciferase and bioluminescence. Subsequently, bioluminescence expression is proportional to the mutagenicity of the agent.

The *Salmonella* mutagenicity assay (Ames assay) is the most widely accepted bacterial assay for the screening and identification of mutagenic compounds [8]. Independent studies have shown a correlation between mutagenicity in the *Salmonella* assay and carcinogenicity in mammals [9]. The assay uses a number of *Salmonella* strains with pre-existing mutations that disable the cells from synthesising histidine, thus inhibiting growth. Fresh mutations at the site of these pre-existing mutations can restore the gene's function and allow the cells to re-synthesis histidine. Enumerations of these mutated colonies in the absence of histidine enable an assessment of mutagenicity [10]. The assay is reliable but laborious and requires working with *Salmonella* strains, which are classified as human pathogen.

In this study, MMC was extracted using a human digestion simulation procedure (the *in vitro* bioassay), and the mutagenicity of the extracted samples was measured using the *Salmonella* assay and SOS-lux based microbial biosensors. This enabled an overall evaluation of the optimised assays, before working with a complex environment like soil. MMC was then amended into soil which extracted using the *in vitro* bioassay, followed by comparative mutagenic assays. Furthermore, the effect of the simulated gastrointestinal constituents on the mutagenicity of MMC was assessed. The aim of this work was to validate the performance of microbial biosensors for mutagenicity assessment and consider the relevance of the in vitro bioassay in assessing pollutant exposure.

Materials and Methods

Stock solutions and soil preparation

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Mutagenicity testing using the *Salmonella* assay and the SOS-*lux* biosensors was performed against a range of doses of mitomycin C (MMC). MMC was purchased from Sigma (St.Louis, MO, USA), and dissolved in reverse osmosis water. Soil (sandy loam) of Insch Association/Insch Series (North-East Scotland) was collected to a depth of 50 cm, passed through a 2 mm stainless steel sieve, thoroughly mixed and amended with MMC to give final concentrations of 1, 10 and 50 µg MMC/g dry weight (dw) soil using standard procedures [11]. Soil amended with MMC was extracted by the *in vitro* digestion extraction and then assayed. The dose selection of MMC for soil amendment was based upon the dose response curves of MMC, which tested with the *Salmonella* assay and the SOS-*lux* biosensors. Un-amended soil (MMC free soil) was used as a negative control.

The *in vitro* bioassay

The sequential and the compartmental *in vitro* digestion extractions were carried out for soils amended with MMC. The sequential *in vitro* digestion assay was performed as described by Oomen et al. [1]. The compartmental approach was performed by extracting soil samples separately in each digestive compartment (i.e. saliva, gastric and duodenal). Synthetic gastrointestinal juices were simulated as described by Oomen et al. [1]. In summary, the saliva extraction was performed by adding 9 ml of synthetic saliva (pH 6.5 ± 0.2) to 0.6 g soil. The mixture was rotated for 5 min using an end-over-end shaker. The samples were centrifuged for 10 min at 1730 g and 37°C ± 2°C. The "digested soil" (the pellet) was discarded and the supernatant (the chyme) removed and stored at 4°C. Gastric extraction was performed by adding 13.5 ml of the synthetic gastric juice (pH 1.1 ± 0.1) to 0.6 g soil, and the mixture was mixed by end-over-end shaking for 2 h, centrifuged and stored at 4°C. The intestinal extraction was carried out by adding 27 ml of the synthetic duodenal juice (pH 7.8 ± 0.2) and 9 ml of the synthetic bile (pH 8.0 ± 0.2) to 0.6 g soil. The mixture was rotated for 2 h and centrifuged as above. The digestive juices were maintained at 37 ± 2°C in a water bath, prior to assay.

The Ames assay

The standard plate incorporation procedure described by Maron and Ames [12] was used for the Ames assay. In brief, *Salmonella* strains TA98, TA100 and TA102 were grown overnight in 150 ml Erlenmeyer flask containing 25 ml Oxoid nutrient broth at 37°C in an orbital shaker at 150 rpm with appropriate antibiotics (25 µg ml^{-1} ampicillin for TA98 and TA100, and 2 µg ml^{-1} tetracycline for TA102). The cultures were incubated until they reached an absorbance of 1.0 at 660 nm (corresponding to 1-2×109 CFU ml^{-1}). Two ml of melted top agar supplemented with histidine and biotin solution was distributed into sterile glass tubes, and placed in a 45°C water bath. A hundred µl of MMC extracted by the *in vitro* bioassay and 100 µl of the tester strain was added, gently mixed by vortexing, and poured onto the surface of Minimal Glucose Agar plate. The plates were gently tilted and rotated to obtain an even distribution, placed onto a level surface to solidify and incubated at 37°C for 48 h. Following the incubation, the revertant colonies were enumerated on a Gallenkamp colony counter. Appropriate reagent and negative controls [12] were included to enumerate the spontaneous revertants.

The assay was conducted using triplicate of each sample and control. *Salmonella typhimurium* TA98, TA100 and TA102 were obtained from Molecular Toxicology Inc. (MD, USA). The strains were maintained and stored according to standard protocols [10].

SOS-*lux* biosensors

Biosensor strains *E. coli* K12C600 and *E. coli* DPD1718 were obtained from Rettberg et al. [7] and Vankemmelbeke et al. [13], respectively. Overnight cultures were grown on LB media at 37°C in an orbital shaker at 150 rpm in the presence of the appropriate antibiotics (50 µg ml^{-1} ampicillin for *E. coli* K12C600 and 30 µgml^{-1} chloramphenicol for *E. coli* DPD1718). Overnight cultures were diluted 1:50 in LB broth, and grown at 37°C, until they reached the appropriate pre-optimised optical density (0.3 at OD_{550} for *E. coli* K12C600 and 0.4 at OD_{492} for *E. coli* DPD1718). A negative control of 100 µl MilliQ water or 100 µl sample was mixed with 900 µl of overnight culture in 3 ml luminometer cuvettes. Bioluminescence was measured using a Jade bench-top luminometer (Labtech International, Uckfield, UK), over a period of 300 min, with readings taken every 30min.

Analytical techniques for MMC

MMC was analytically measured after the *in vitro* digestion extractions carried out for soils amended with MMC. The method adopted was modified from Metha et al. [14]. Soil samples (2 g ± 0.01) were grounded with 2 g of anhydrous sodium sulphide. Samples from the *in vitro* digestion assay were transferred to 30 ml Wheat on vials and 15 ml of methanol was added. The samples were sonicated, and for 6 h placed on an over and under shaker. Samples were centrifuged at 4°C for 10 min in glass centrifuge tubes. The sample was filtered through alumina and transferred to 1.5 ml HPLC sealed glass vials for analysis. Analysis was performed at 365 nm by HPLC (Thermoquest; Thermo Separation Products, San Jose, CA) on an octadecylsilicate column (15 cm 4.6 mm, i.e. particle diameter 5 ml), at a flow rate of 1.0 ml min^{-1}.

The mobile phase was an acetonitrile: water mix with an elution gradientof 30:70 initially for 10 min; then increased to 60:40, over 10 min, and finally increased to 90:10 over 5 min.

Statistical analysis

Each sample was tested in triplicate. Statistical analysis was performed using Minitab 15 for Windows. A result with $p \leq 0.05$ was considered significant. The two fold increase rule was applied for the *Salmonella* assay to evaluate the mutagenicity of the tested compounds [12]. For the biosensors, a compound was considered a mutagen if there was at least a two fold increase in the bioluminescence response relative to the negative control value [15,16]. If the bioluminescence values decreased during the incubation time, the sample was more likely to be cytotoxic [7]. If bioluminescence was not induced and the cell growth was comparable to that of the untreated control, the test sample was assumed to be neither mutagenic nor cytotoxic.

Results

Mutagenicity response of the *Salmonella* assay to MMC tested in the aqueous phase

Salmonella strain TA102 was the most sensitive strain to detect the mutagenicity of MMC (Table 1). *Salmonella* strains TA98 and TA100 were, by comparison, insensitive to MMC, as the numbers of histidine revertants were not twice the numbers of the spontaneous revertants (Table 1). A significant mutagenic response was detected for MMC tested with TA102, and extracted in the saliva and the duodenal compartments. There was no significant mutagenic response detected for MMC extracted in the gastric and in the sequential compartments (Table 1). To further investigate the reason for that, the pH of the two compartments were buffered, and the mutagenicity was re-evaluated

Sample	Dose (µg/Plate)[B]	Number of revertants/plate (mean ± SE)[D]		
		TA98	TA100	TA102
Saliva	0[C]	***38 ± 4***[E]	***151 ± 9***[E]	***449 ± 29***[E]
	0.5	4 ± 1	0 ± 0	1836 ± 57[F]
	1	1 ± 0	13 ± 1	2098 ± 155[F]
Positive control[A]		**1675 ± 0[F]**	**484 ± 29[F]**	**2151 ± 95[F]**
Gastric	0[C]	***38 ± 4***[E]	***151 ± 9***[E]	***449 ± 29***[E]
	0.5	42 ± 3	61 ± 9	502 ± 21
	1	44 ± 5	84 ± 4	468 ± 15
Positive control[A]		**1675 ± 0[F]**	**484 ± 29[F]**	**2151 ± 95**
Duodenal + Bile	0[C]	***38 ± 4***[E]	***151 ± 9***[E]	***449 ± 29***[E]
	0.5	9 ± 2	13 ± 2	1800 ± 74[F]
	1	0 ± 0	54 ± 5	1699 ± 116[F]
Positive control[A]		**1675 ± 0[F]**	**484 ± 29[F]**	**2151 ± 95[F]**
Sequential	0[C]	***38 ± 4***[E]	***151 ± 9***[E]	***449 ± 29***[E]
	0.5	37 ± 1	166 ± 5	524 ± 31
	1	38 ± 3	170 ± 10	473 ± 19
Positive control [A]		**1675 ± 0[F]**	**484 ± 29[F]**	**2151 ± 95[F]**

[A]Positive Control: TA98-Daunomycin (6 µg/Plate); TA100-Sodium Azide (1.5 µg/Plate); TA102-Mitomycin C (0.5 µg/Plate). [B]Concentration based on 100×15-mm Petri dish containing 20 to 25 ml of MG agar. The doses of the tested mutagens were expressed as µg/plate (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). [C]Negative control: Filter sterilised water. [D]Number of histidine revertants per plate: Mean values of at least three plates ± standard error (SE). [E]Numbers in italic, boldface and underlined represent the number of spontaneous revertants colonies for: TA98 (30-50), TA100 (120-200) and TA102 (117-530) (Maron and Ames, 1983). [F]Numbers in boldface represent two fold increase or more in the number of revertant colonies over the solvent controls (spontaneous revertants), which was an indication of a significant mutagenic response.

Table 1: The number of reverse mutants of S. typhimurium TA98, TA100 and TA102 after exposure to MMC Samples were analysed in the aqueous phase after extraction by the *in vitro* procedure.

Sample	Dose (µg/Plate)	Number of revertants/plate (mean ± SE)[C]
Gastric (pH 1.07)	0[B]	***467 ± 12***[D]
	0.5	505 ± 20
	1	424 ± 8
Positive control[A]		1223 ± 117[E]
Gastric (pH 7.4)	0[B]	***467 ± 12***[D]
	0.5	510 ± 11
	1	***493 ± 27***
Positive control[A]		1223 ± 117[E]
Sequential (pH 5.8)	0[B]	***467 ± 12***[D]
	0.5	507 ± 8
	1	***475 ± 27***
Positive control[A]		1223 ± 117[E]
Sequential (pH 7.5)	0[B]	***467 ± 12***[D]
	0.5	511 ± 12
	1	480 ± 5
Positive control[A]		1223 ± 117[E]

[A]Positive Control for TA102- Mitomycin C (0.5 µg/plate). [B]Negative control: Filter sterilised water. [C]Number of histidine revertants per plate: Mean values of at least three plates ± standard error (SE). [D]Numbers in italic, boldface and underlined represent the number of spontaneous revertants colonies for TA102 (117-530). [E]Numbers in boldface represent two fold increase or more in the number of revertant colonies over the solvent controls (spontaneous revertants), which was an indication of a significant mutagenic response.

Table 2: The number of reverse mutants of *S. typhimurium* TA102 after exposure to MMC Samples were analysed in the aqueous phase after extraction by the *in vitro* procedure (buffered and un-buffered samples).

with TA102 (Table 2). The pH values of samples extracted in the gastric juice were increased from 1.07 ± 0.10 to 7.4 ± 0.10. Similarly, the pH values were increased from 6.8 ± 0.20 to 7.5 ± 0.10 for samples extracted by the sequential *in vitro* bioassay. There was no significant mutagenic response for MMC extracted in the gastric, and in the sequential compartments for the buffered (pH values 7.4 ± 0.10 and 7.5 ± 0.10) and un-buffered (pH values 1.07 ± 0.10 and 6.8 ± 0.20) samples (Table 2).

Mutagenicity response of SOS-*lux* biosensors toMMC tested in the aqueous phase

The biosensor (*E. coli* K12C600) dose response for the negative control (MilliQ water) was significantly different to the samples treated with MMC and extracted in the saliva and the duodenal compartments (Figure 1). The biosensor *E. coli* K12C600 was not significantly induced by samples treated with MMC and extracted by the gastric and the sequential *in vitro* bioassay (Figure 1). These results suggested that the biosensor *E. coli* K12C600 was induced by MMC extracted in the saliva and in the duodenal compartments, whereas MMC extracted by the gastric and the sequential *in vitro* bioassays was no longer a mutagen, as it was not able to induce *E. coli* K12C600 (Figure 1). The same results were observed for *E. coli* DPD1718 (Figure 2) (saliva and in the duodenal compartments, but not the gastric and in the sequential compartments). These findings were compatible with the results reported with the biosensor *E. coli* K12C600, suggesting that MMC was not a mutagen, once it passed through the gastric compartment of the simulated human gastrointestinal juices (Figures 1 and 2).

Mutagenicity response of the *Salmonella* assay to MMC amended soil

The *Salmonella* mutagenicity assay responded significantly to Insch soils amended with 10 and 50 µg MMC/g dw soil (Table 3). For Insch soil extracted with the gastric and the sequential *in vitro* extractions (Table 3), no significant mutagenic response was observed. The *Salmonella* results confirmed that MMC had a mutagenic effect at a concentration of 10 µg MMC/g dw soil and above, when extracted with synthetic saliva and duodenal juices. At the concentrations tested, MMC had no mutagenic impact on the *Salmonella* strain TA102 in the sequential and the gastric *in vitro* extractions due to the denaturing of MMC at the extraction pH value (Table 3).

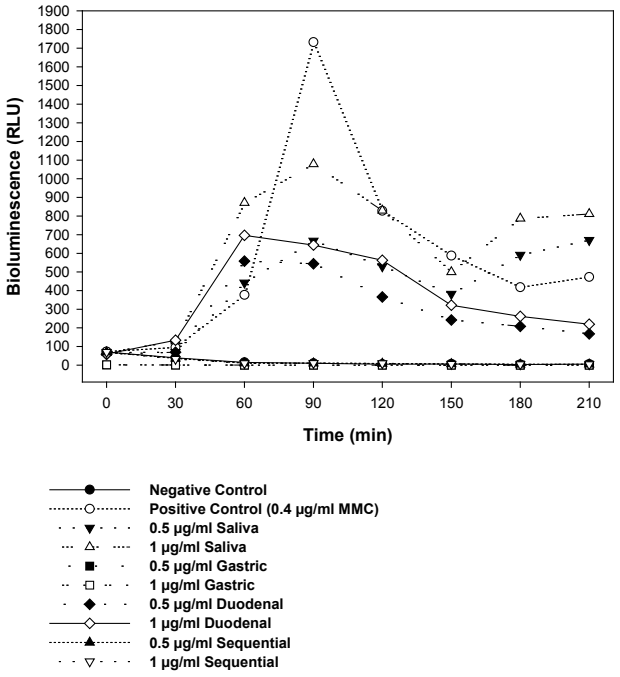

Figure 1: Mutagenicity response of the SOS-*lux* microbial biosensor *Escherichia coli* K12C600 to MMC in the aqueous phase and extracted by the *in vitro* extraction. The biosensor was only induced by samples treated with 0.5 and 1µg/ml MMC, and extracted in the saliva and the duodenal compartments.

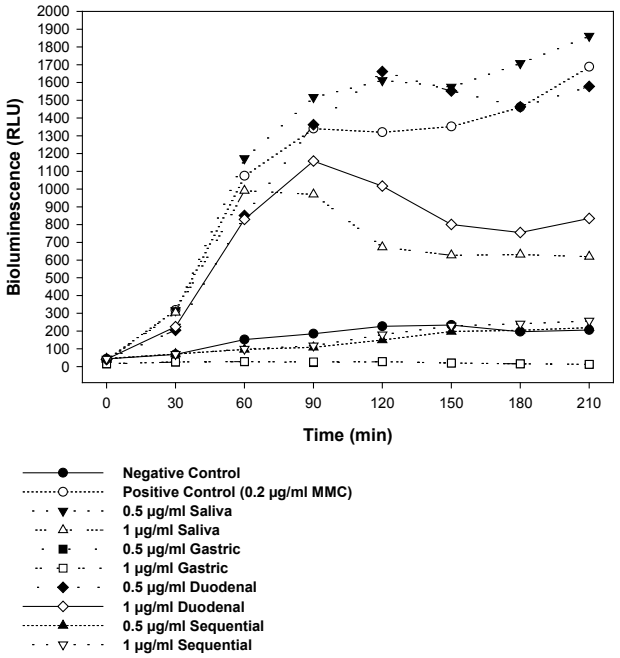

Figure 2: Mutagenicity response of the SOS-*lux* microbial biosensor *Escherichia coli* DPD1718 to MMC tested in the aqueous phase and extracted by the *in vitro* extraction. The biosensor was only induced by samples treated with 0.5 and 1 µg/ml MMC, and extracted in the saliva and the duodenal compartments.

Gastrointestinal Compartments	Dose µg/g dry weight soil	Number of revertants/ plate (mean ± SE)[D]
Saliva	0[B]	***314 ± 2***[E]
	0[C]	450 ± 39
	1	477 ± 4
	10	646 ± 1[F]
	50	950 ± 0[F]
Positive Control[A]		1073 ± 5[F]
Gastric	0[B]	***314 ± 2***[E]
	0[C]	390 ± 7
	1	394 ± 4
	10	417 ± 2
	50	381 ± 9
Positive Control[A]		**1073 ± 5**[F]
Duodenal + Bile	0[B]	***314 ± 2***[E]
	0[C]	356 ± 5
	1	374 ± 60
	10	687 ± 101[F]
	50	956 ± 0[F]
Positive Control[A]		1073 ± 5[F]
Sequential	0[B]	***314 ± 2***[E]
	0[C]	393 ± 6
	1	419 ± 4
	10	428 ± 4
	50	440 ± 15
Positive Control[A]		**1073 ± 5**[F]

[A]Positive Control for TA102-Mitomycin C (0.5 µg/plate). [B]Negative control: Filter sterilised water. [C]Negative control for each gastrointestinal compartment (i.e. saliva, gastric, duodenal and sequential). These samples contained un-amended soils (soils without MMC). [D]Number of histidine revertants per plate: Mean values of at least three plates ± standard error (SE). [E]Numbers in *italic*, boldface and underlined represent the number of spontaneous revertants colonies for TA102 (117-530). [F]Numbers in boldface represent two fold increase or more in the number of revertant colonies over the solvent controls (spontaneous revertants), which was an indication of a significant mutagenic response.

Table 3: The number of reverse mutants of *S. typhimurium* TA102 after exposure to Insch soils amended with MMC and extracted by the sequential and compartmental (saliva, gastric and duodenal) *in vitro* extraction.

Mutagenicity response of SOS-*lux* biosensors to MMC amended soil: For soil amended with MMC and extracted in the oral compartment, a significant mutagenic response was measured at concentrations of 1, 10 and 50 µg MMC/g dw soil for *E. coli* DPD1718 (Figure 3) and *E. coli* K12C600 (Figure 4). Significant mutagenic responses to 10 and 50 µg MMC/g dw soil were reported for soil extracted in the intestinal compartment and assayed with *E. coli* DPD1718 (Figure 3) and *E. coli* K12C600 (Figure 4). The biosensors *E. coli* DPD1718 and *E. coli* K12C600 were not significantly induced by MMC in the gastric and the sequential *in vitro* extractions from Insch soils because MMC denatured at these pH values (Figures 3 and 4). MMC was more likely to be destroyed because of the acidic pH of the gastric compartment rather than the gastric enzymes. If the gastric enzymes should have an effect on MMC, this effect should be obvious with the saliva and the duodenal compartments, as both of them have enzymes such as amylase, lipase and pancreatin.

HPLC results for soils amended with MMC: The pH values of the simulated gastrointestinal compartments had a significant influence ($p<0.05$) on the measured concentrations of MMC. The concentrations of MMC were 0.69, 8.50, and 49.01 µg MMC/g dw soil for soil amended with 1, 10 and 50 µg MMC/g dw soil, respectively, and extracted in the saliva. For the duodenal compartment, the measured concentrations of MMC were 0.90, 6.32 and 48.32 µg MMC/g dw soil. These results confirmed that the pH values of the saliva and the duodenal had no

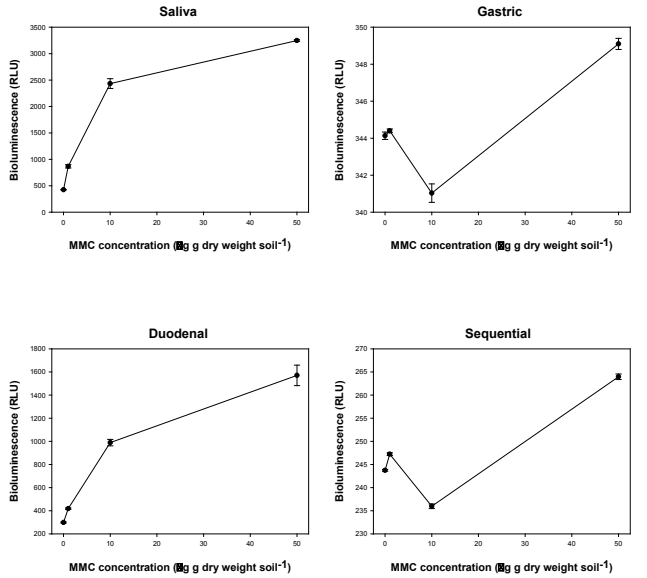

Figure 3: Mutagenicity response of the SOS-*lux* microbial biosensor *Escherichia coli* DPD1718 to Insch soils amended with MMC and extracted by the sequential and the compartmental (saliva, gastric and duodenal) *in vitro* extraction. Error bars represent the standard errors of the mean of triplicate measurements.

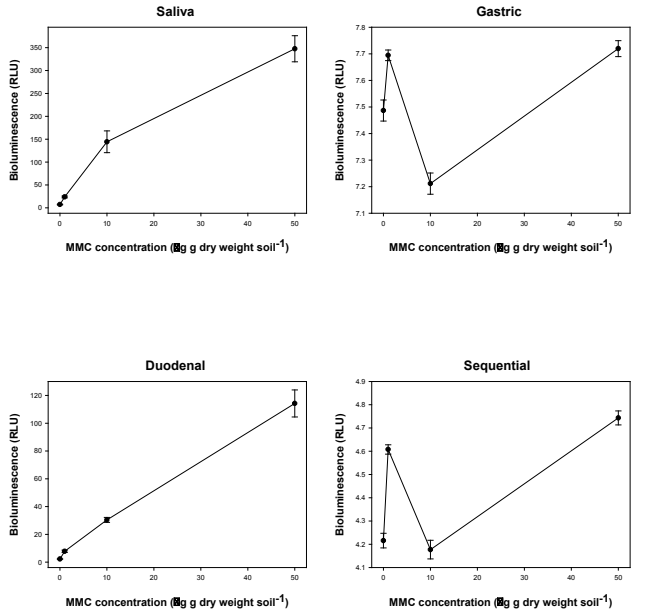

Figure 4: Mutagenicity response of the SOS-*lux* microbial biosensor *Escherichia coli* K12C600 to Insch soils amended with MMC and extracted by the sequential, and the compartmental (saliva, gastric and duodenal) in vitro extraction. Error bars represent the standard errors of the mean of triplicate measurements.

significant effect on the measured concentrations of MMC. The minute reduction in the measured concentrations of MMC was due to the low pH value (4.2 ± 0.0) of Insch soil [11]. For soil extracted in the gastric compartment, the measured concentrations of MMC were 0.35, 0.21, and 0.37 µg MMC/g dw soil for soils amended with 1, 10 and 50 µg MMC/g dw soil, respectively. Similar results were reported for soil extracted in the sequential compartment (the measured concentrations of MMC were 0.50, 0.34 and 0.18 µg MMC/g dw soil for soils amended with 1, 10 and 50 µg MMC/g dw soil, respectively). The acidic pH of the gastric compartment (1.1 ± 0.1), and Insch soil (4.2 ± 0.0) destroyed MMC and caused an enormous reduction in the measured concentrations of MMC.

Discussion

MMC was selected for this study because it is a highly water soluble chemical acknowledged to induce a response both to the Ames assay and the biosensor [17]. MMC is a natural occurring compound consisting of a pyrrolo (1,2-a) indole ring system with an aziridine ring.MMC is a potent DNA cross-linker, which has a strong ability to crosslink DNA with high efficiency and specificity for the sequence CpG [18]. To interact with DNA, MMC requires enzymatic activation by a one-electron pathway to a semiquinone, or by a two-electron reduction pathway to a hydroquinone [19]. MMC has several biological effects in mammalian cells, such as mutagenesis, stimulation of genetic recombination, selective inhibition of DNA synthesis, chromosome breakage and sister chromatid exchange and induction of the DNA repair system (SOS-response) [18]. The anti-cancer activity of MMC is based upon its covalent binding to DNA after chemical or enzymatic reductive activation [20].

Salmonella strain TA102 was the most sensitive among the *Salmonella* strains to detect the mutagenicity of MMC. Growth inhibition was observed with the *Salmonella* strains TA98 and TA100 when exposed to MMC (Table 1). The same results were also reported by Maron and Ames [12], who observed inhibition of growth with TA97, TA98 and TA100 when exposed to 2.5 µg/plate of MMC. Consequently, MMC was adopted as a positive control to assess the number of histidine revertants colonies for TA102 [10,12,21]. There is no doubt that the biosensor yielded rapid results when compared with the Ames assay, and this is widely acknowledged [22,23]. An acknowledged limitation of biosensors is that future developments will require the characterisation and adoption of strong promoters offering genuine relevance with key target receptors [24].

The selection of the particular assay procedures reported was because these were acknowledged to be responsive to MMC, but translation to other chemicals of concern (particularly hydrophobic compounds such as PAHs) may require a new suite of mutagenic responsive sensors and assays. In this study, the biosensor performance and sensitivity was similar to the Ames assay. This has been used to reinforce the merit of rapid screening assays to derive values for human protection. But there is a need to take a wider view. The main challenge is that the data that underpin the human protective strategy must be validated against relevant receptors. Comparing biosensor or Ames derived data with those from animal experiments where injection of the chemical into the animal has occurred may be of limited value. So, before the adoption of protective values or the use of routine screening approaches, users must be confident about the relevance of the adopted data.

The impact of soil borne mutagenic compounds on human receptors is of considerable interest when deriving threshold values that will prove protective [3]. There would be little relevance in simply using a solvent extraction procedure to collect the analyte and then measure the response, because this would not reflect the human bioaccessible fraction [1]. For MMC, there were problems with the stage of the assay procedure that mimicked the conditions of the stomach. The acidic nature of this step oxidised the test compound, and this is likely to

be a problem with other chemicals of concern. A mutagenic response to MMC was recorded only for soil samples extracted in the oral and the intestinal compartments (pH 6.5 ± 0.2 and 7.8 ± 0.2, respectively) (Table 3 and Figures 3 and 4). There was no mutagenic response to MMC by the *Salmonella* assay or the SOS-*lux* biosensors for the gastric or the sequential *in vitro* digestion assay, because the compound had been destroyed under the acidic condition (pH 1.1 ± 0.1) of the gastric compartment. Even after a buffering step to mediate the pH value, there was no significant mutagenic response, which was confirmed by chemical analysis (Table 2). This concentration should not pose an unacceptable risk to a child ingesting up to 0.6 g of soil daily for six years contaminated with MMC.

The particular relevance and indeed the focus of this work was that it combined a human bioaccessibility assay with mutagenic testing. Such *in vitro* assays were developed for inorganic chemicals of concern, but there has been wider translation to other pollutants and mixed contaminants. This bioaccessibility *in vitro* procedure verifies a relevant environmental interface because this considers the potential pathways through which humans could become exposed to such mutagenic compounds. The question is not about the mutagenicity of soil, but is about the mutagenic nature of compounds that are bioaccessible in the soil. A constraint however, was that a specific stage of the *in vitro* assay oxidised the analyte. Further refinement of this procedure to adopt less harsh surrogate steps for the stomach and duodenal stage could mitigate this, but detailed correlation would be required against established data.

A key advantage of the application of biosensors is their ability to be deployed to genuine samples [24]. This means that while chromatographic analysis requires extraction, purification, clean-up, potentially derivatisation and then analysis, the biosensor can be used in crude and turbid solutions. This could be exclusively for a mutagenic sensor or for a wider suite of sensors for specific analytes and generic toxicity. Samples could be exposed to a suite of sensors simultaneously, allowing a real time response that could prove valuable for the protection of target receptors at a timescale commensurate with intervention [22]. The sensors could also be fabricated to permit compact analysis and the use of disposable electrodes. Such technologies could transform routine testing and interpretation.

Conclusion

This study enabled an empirical assessment of a model mutagen borne in soil and its potency on human receptors.

SOS-*lux* biosensors had numerous practical advantages over the traditional assays, including procedural simplicity, a rapid and unambiguous result, ease of measurement, rapid tabulation of exposure concentration and *in vivo* analysis without cell disruption. A critical insight for the bacterial SOS system and the regulatory pathway, which is responsible for inducible DNA repair system, is essential for the genetic engineering of these biosensors. This engineering must be placed in parallel with environmental applicability that enables the collection and analysis of relevant environmental samples and complementary chemical characterisation. As mutagenic-based biosensors evolve, the key traits of this study can be translated to ensure relevance and applicability.

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