CYP1A1 and Cnr nitroreductase bioactivated niclosamide in vitro

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Niclosamide produces genotoxic effects, such as point mutations in Salmonella sp., sperm-head abnormalities in mice and clastogenic effects in human lymphocytes in vitro and in vivo. As cytochrome P450 could be involved in the bioactivation of niclosamide, we investigated which sub-family was involved. We used liver microsomal fractions from rats treated with phenobarbital/β-naphthoflavone (PB/β-NF), benzo[a]pyrene (BaP) or cyclohexanol, which are known to induce different cytochrome P450 subfamilies, such as CYP2B, CYP1A1, CYP1A2 and CYP2E1. We also inhibited CYP1A and CYP2E using α-NF and diethyl-dithiocarbamate to identify the cytochrome P450 involved. Liver-S9 fractions obtained from PB/β-NF- and BaP-treated rats significantly increased the number of revertants induced by niclosamide, while the CYP1A1 inhibitor α-NF decreased the number of revertants. The incubation of niclosamide with CYP1A1 Supersomes™ increased the number of revertants, suggesting that CYP1A1 is responsible for the bioactivation of niclosamide. Nitroreduction is also involved in the bioactivation of niclosamide, as the nitroreductase-deficient strain YG7132 did not respond to the niclosamide treatment. Our findings indicated that a metabolite, derived from the action of CYP1A1 and a nitroreduction-reaction process, has a key role in the bioactivation of niclosamide.

Introduction
Niclosamide (5-chloro-N-[2-chloro-4-nitrophenyl]-2-hydroxy-benzamide) has a broad use ranging from pest management to medicine. It is commonly used in treating infections produced by trematodes and cestodes in human and non-human organisms (1,2) and for pest control, including molluscs (3). Niclosamide also has been used as an effective treatment against intestinal tapeworm infections (4) and as an effective drug to inhibit the synthesis of coronavirus proteins in severe acute respiratory syndrome in Vero cells (5). Niclosamide is also of interest to researchers because it might be used as a treatment for cancer. This interest is supported by the finding that niclosamide inhibited S100A4-induced metastasis formation in a mouse model of colon cancer (6) and inhibited Wnt/Frizzled1 signalling in human colon cancer cell lines and colorectal cancer cells (7,8). In addition, niclosamide induced apoptotic and autophagic cell death in HeLa cells (9). Moreover, some evidence has indicated that niclosamide damages DNA. For example, niclosamide-treated mice showed an increase in the number of sperm-head abnormalities (10). Niclosamide also produced clastogenic effects in human lymphocytes in vitro and in vivo (11) and frame-shift mutations in Salmonella sp. (12). Importantly, the mutagenic effect of niclosamide in vitro depends on the presence of the liver-S9 fraction, which incorporates phase I enzymes (13), including cytochrome P450 (CYP). In most cases, a loss in the biological activity of the substrate results from the participation of phase I enzymes. These metabolic reactions are considered detoxification pathways (14). However, the biotransformation of some xenobiotics leads to the formation of reactive metabolites involving oxidation and reduction reactions (15,16).

CYPs play a crucial role in the biosynthesis and metabolism of a number of endogenous substrates (e.g. steroid hormones and lipophilic signal molecules) and in the detoxification or activation of a variety of xenobiotics (17). CYPs have been implicated in the bioactivation of many substances, including pesticides (18). CYP2E1, CYP2B1, CYP1A1/2 and CYP2C11 are important CYPs that are involved in the bioactivation of xenobiotics in rat lung and liver tissues (19). Other CYPs are known to play relatively minor roles in the metabolic activation of procarcinogens and promutagens.

Nitroreduction is also involved in the bioactivation of various compounds, such as nitroaromatic hydrocarbons or nitroarenes (20,21). This process forms nitrenium ions, which might be responsible for the mutations involved in carcinogenesis (22,23), such as DNA-strand breaks (24). Niclosamide mutagenicity seems to depend on nitroreduction (12), and there is evidence suggesting that this compound could even interact with DNA following reductive activation (25).

Despite the insight that we have gained into the effects of niclosamide, its bioactivation pathway has not been fully elucidated. Studying the bioactivation pathway of niclosamide will add to the understanding of the mechanism of action and its possible interaction with other xenobiotics including drugs, food components or environmental contaminants. Previous reports have suggested that CYPs and nitroreductases participate in the bioactivation of niclosamide; therefore, this study aimed to identify the enzymes involved in this bioactivation. First, we confirmed the mutagenic and toxic effects of niclosamide using the Ames test. Second, we examined (i) the effect of different liver-S9 fractions prepared from rats treated with known CYP inducers; (ii) the effect of CYP inhibitors and recombinant CYPs on the bioactivation of niclosamide; and (iii) the participation of Cnr nitroreductase in the bioactivation of niclosamide.

Materials and methods

Animals
For CYP induction, 15 male Wistar rats aged 9–10 weeks were used. The rats were obtained from the animal facility of the Instituto de Investigaciones Biomédicas and housed in groups of four in plastic cages. The rats were placed in a light- and temperature-controlled environment with ad libitum access to food and water. The experiments, which were conducted in accordance with the ethical guidelines for investigation with laboratory animals, were approved by an ethical committee for animals at the Instituto de Investigaciones Biomédicas.

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We used the following Salmonella typhimurium strains for the Ames test: TA98, YG1020, YG1021, YG1024 and YG7132 whose genotypes were previously described (26–29). Each strain was designed to be responsive to frame-shift mutations. The strains were maintained as described by Maron and Ames (30).

**Chemicals**

We used the following chemicals for the experiments: niclosamide, β-nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate, L-histidine, D-biotin, D-glucose, potassium phosphate, magnesium sulphate, benzo[a]pyrene (BaP), 1-nitropyrene, α-naphthoflavone (α-NF) and dimethyl sulfoxide (DMSO). These chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA). We also used nutrient broth No. 2 (Oxoid Inc., Ogdensburg, NY, USA), 2-aminoantracene (2-AA; Chemical Company, Milwaukee, WI, USA), nitrofurantoin, N-nitrosopyrrolidine and diethylthiobis(μ-oxo)octamericum (DDTC Sigma-Aldrich). The solvents used in this study were of a high analytical grade. Supersomes™ were obtained from Becton Dickinson (Gentest Co. Woburn, MA, USA). Supersomes™ are microsomes from insect cells transfected with complementary DNAs encoding for rat CYP1A1 and CYP2B1, along with NADPH-CYP reductase and cytochrome b5.

**Animal treatment**

The procedure for treating the rodents with enzyme inducers to increase the expression of CYP1A1, CYP2B and CYP2E is described elsewhere (31,32). For this purpose, the rats were randomly assigned to three groups (five rats/group) and treated with either BaP, phenobarbital (PB)/β-NF or cyclohexanol (CH). The enzyme inducers were diluted in corn oil and administered intraperitoneally in a volume of 200 μl, except for CH, which was diluted in drinking water. The first group of rats received 60 mg/kg of PB for 3 days followed by 30 mg/kg of PB and 80 mg/kg of β-NF on the fourth day. The second group received 20 mg/kg of BaP, the third group received 25% vol/vol of CH in drinking water, which was available ad libitum for 5 days. The rats were euthanised 24 h after the last treatment day, and their livers were obtained aseptically.

**Mutagenicity test**

Experiments of niclosamide bioactivation by S9 fractions prepared from rats treated with known CYP inducers were conducted using the standard plate incorporation method of Ames test as described elsewhere (30,33). Niclosamide was dissolved in DMSO. 1-Nitropyrene (0.01 μg/μl), BaP (5 μg/μl), N-nitrosopyrrolidine (200 μg/μl) and 2-AA (5 μg/μl) were used as positive controls and were dissolved in DMSO or water, depending on their solubility.

In the following experiments, we used the pre-incubation method (30), (i) inhibition of niclosamide bioactivation; (ii) bioactivation of niclosamide with Supersomes™; involvement of nitroreduction in niclosamide bioactivation and (iv) genotoxicity of aminoniclosamide in the presence of liver-S9 fraction. All reaction mixtures were incubated for 30 min at 37°C in a shaking bath, and 2 ml of molten top agar were added and distributed in the minimal agar plates. The plates were incubated at 37°C for 48 h, and the number of revertant colonies was counted.

**Reduction of niclosamide nitro group**

The nitroreduction method for niclosamide was adapted from the procedure used by Khier et al. (34). Briefly, the reduction was obtained by dissolving niclosamide in a 1 N hydrochloric acid solution to which 2 g of zinc dust was added; the solution was occasionally shaken. The reaction was allowed to stand for 1 h at room temperature, and then it was filtered; its product was recrystallised. The synthesized aminoniclosamide was dissolved in 1 N hydrochloric acid solution to which 2 g of zinc dust was added; the solution was occasionally shaken. The reaction was allowed to stand for 1 h at room temperature, and then it was filtered and then dried following TLC development, and its product was recrystallised. The solvents used in this study were of a high analytical grade. Supersomes™ were obtained from Becton Dickinson (Gentest Co. Woburn, MA, USA). Supersomes™ are microsomes from insect cells transfected with complementary DNAs encoding for rat CYP1A1 and CYP2B1, along with NADPH-CYP reductase and cytochrome b5.

**Results**

**Mutagenic effect of niclosamide in the presence of the liver-S9 fraction**

The liver-S9 fraction contributed significantly to the total variation in the numbers of revertant colonies (Table I). The average number of revertants in the absence of the liver-S9 fraction (8.58 ± 2.41, n = 24) was statistically lower than in the presence of the liver-S9 fraction (51.83 ± 10.44, n = 24, Wald Z = 3.18, P = 0.001). Niclosamide concentrations >5 μg/plate totally reduced the average number of revertants in the absence of the liver-S9 fraction (Table II). Conversely, the liver-S9 fraction allowed the number of colonies to grow, although the concentrations of niclosamide >15 μg/plate did not hinder the liver-S9 fraction from protecting the bacteria (Table II). The general difference in the manner the bacteria responded to niclosamide in the absence and presence of the liver-S9 fraction was explained by the interaction term (Table I).

**Cytochrome P450 contributes to the bioactivation of niclosamide**

As the liver-S9 fraction incorporates most of phase I enzymes, we investigated whether CYPs were involved in the bioactivation of niclosamide. Both the niclosamide and liver-S9 fractions from rats previously treated with either CH, PB/β-NF or BaP significantly affected the average number of revertants (Table I), but as the exclusion of the interaction term did not change the variance of the model (Table I), the slopes (i.e. mutagenic potential) did not differ among the enzyme inducers (Figure 1). However, the average size of the effect varied. The effect of CH (111.72 ± 8.74, n = 18) was significantly smaller than that of PB/β-NF (177.88 ± 16.64, n = 18; Z = −4.72, P = 0.000) and BaP (201.61 ± 18.13, n = 18; Z = 4.46, P = 0.000). Altogether, these results suggested that the CYP1A1 and most likely CYP2B subfamilies might be involved in the bioactivation of niclosamide.

**Inhibition of the bioactivation of niclosamide**

To determine which CYP was associated with the bioactivation of niclosamide, we used α-NF, which inhibits both CYP1A1 and CYP1A2. The results showed that concentrations of 100–150 μM of α-NF significantly reduced the average number (~50%) of BaP-induced revertants (Z = 9.08, n = 18, P = 0.000; Figure 2a), and concentrations of 175–200 μM reduced this number even more (~65%; Z = 12.44, n = 12, P = 0.000; Figure 2a). Similarly,
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Table I. Statistical summary of model simplification of the fit of generalised linear mixed models to the data of the bioactivation of niclosamide as a function of the number of revertants

| Step* | Simplification | df | logLik | $\chi^2$ | P-value |
|-------|----------------|----|--------|----------|---------|
| a)    | Maximal model  |    |        |          |         |
|       | Niclo:S9 (PB/β-NF) | 39 | −268.67|          |         |
|       | Niclo+89 (PB/β-NF) | 32 | −184.88| 83.790  | 0.000   |
| b)    | Maximal model  |    |        |          |         |
|       | Niclo:S9 (PB/β-NF, BaP CH) | 7  | −271.29|          |         |
|       | Niclo+S9 (PB/β-NF, BaP CH) | 5  | −273.95| 5.304   | 0.070   |
|       | Niclo          |    |        |          |         |
| c)    | Maximal model  |    |        |          |         |
|       | α-NF (a, b, c, d, e, f) | 8  | −200.20| 0.004   | 0.949   |
|       | α-NF (a, b, cd, e, f) | 7  | −200.20| 0.004   | 0.949   |
|       | α-NF (a, b, c, e, f) | 6  | −200.79| 0.004   | 0.949   |
|       | α-NF (a, bcde, f) | 5  | −201.59| 0.005   | 0.949   |
|       | Intercept      | 3  | −231.84| 0.005   | 0.949   |
| d)    | Maximal model  |    |        |          |         |
|       | α-NF (a, b, c, d, e, f) | 8  | −194.50|          |         |
|       | α-NF (a, bc, d, e, f) | 7  | −194.53| 0.005   | 0.949   |
|       | α-NF (a, bcd, e, f) | 6  | −194.74| 0.005   | 0.949   |
|       | α-NF (a, bcd, ef) | 5  | −195.18| 0.005   | 0.949   |
|       | Intercept      | 3  | −216.56| 0.005   | 0.949   |
| e)    | Maximal model  |    |        |          |         |
|       | Supersome™1A   | 6  | −97.71 |          |         |
|       | Intercept      | 3  | −130.51| 0.005   | 0.949   |
| f)    | Maximal model  |    |        |          |         |
|       | Supersome™2B   | 7  | −107.21|          |         |
|       | Intercept      | 3  | −133.53| 0.005   | 0.949   |
| g)    | Maximal model  |    |        |          |         |
|       | Strain (YG1021, YG7132); Niclo | 8  | −152.56|          |         |
|       | Strain (YG1021, YG7132)+Niclo | 6  | −179.83| 0.005   | 0.949   |
| h)    | Maximal model  |    |        |          |         |
|       | Aminoniclo (g, h, i, j) | 3  | −87.83 |          |         |
|       | Aminoniclo (g, hi, j) | 5  | −88.13 | 0.005   | 0.949   |
|       | Aminoniclo (g, hi, j) | 4  | −88.51 | 0.005   | 0.949   |
|       | Intercept      | 3  | −95.39 | 0.005   | 0.949   |

Plus and colon denote, respectively, inclusion of an explanatory variable and interaction in the model. P-values refer to the increase in deviance when the respective variable in the model was removed. Lowercase letters refer to treatment levels (a, 0 μM; b, 100 μM; c, 125 μM; d, 150 μM; e, 175 μM; f, 200 μM; g, 0 μg; h, 5 μg; i, 7.5 μg; j, 10 μg) and those that appear together were combined because they did not differ from each other.

*(a) Presence or absence of the liver-S9 fractions, (b) different liver-S9 fractions, (c) inhibition of BaP mutagenicity; (d) inhibition of niclosamide mutagenicity; the effect of (e) CYP1A1 Supersome™; (f) CYP2B1 Supersome™; (g) strains and (h) aminoniclosamide.

Table II. Mutagenicity* of niclosamide in S. typhimurium TA98 without or with liver-S9 fraction

| Niclosamide (μg/plate) | Revertants/plate without S9 (mean ± SEM) | Revertants/plate with S9 (mean ± SEM) |
|------------------------|------------------------------------------|--------------------------------------|
| 0                      | 26.66±3.17                              | 52±6.08                              |
| 1                      | 23±4.16                                  | 38±7.36                              |
| 5                      | 19±1                                     | 105±3.95                             |
| 10                     | T                                        | 121±7.05                             |
| 15                     | T                                        | 98±27.53                             |
| 20                     | T                                        | T                                    |
| 25                     | T                                        | T                                    |
| 30                     | T                                        | T                                    |

*Plate-incorporation method.
Total absence of background lawn. BaP (5 μg/plate) was used as positive control with S9 (415 revertants/plate).

Contribution of a single cytochrome P450 in the bioactivation of niclosamide

The CYP1A1 Supersome™ contributed significantly to the total variation in the average number of revertants (Table I). Niclosamide incubated with 1 μg of CYP1A1 Supersome™ produced a significant increase ($Z = 2.88$, $P = 0.004$) in the average number of niclosamide-induced revertants (56.50±4.99, $n = 6$) compared to the average number of spontaneous revertants (41.83±2.94, $n = 6$; Figure 2a). As expected, 2.5 and 5 μg of CYP1A1 Supersome™ also increased the average number of revertants (113.66±10.17, $n = 6$, $Z = 10.26$, $P = 0.004$) in the average number of revertants (41.83±2.94, $n = 6$; Figure 2a). In contrast, only 150 μg of CYP2B1 produced a significant increase ($t$-test, $t_{(6)} = 3.23$, $P = 0.009$) in the average number of revertants (64.16±6.20, $n = 6$) compared to the average number of spontaneous revertants (42.5±2.23, $n = 6$; Figure 2b).

Nitroreductase promotes the bioactivation of niclosamide in the presence of the liver-S9 fraction

The results showed that the Salmonella strain YG1021 increased the average number of revertants in response to the treatment with 5 μg (179.5±7.28, $n = 6$, $Z = 13.93$, $P = 0.000$) and 7.5 μg of niclosamide (280.83±16.82, $n = 6$, $Z = 17.75$, $P = 0.000$; Figure 4). In contrast, the nitroreductase-deficient strain YG7132 did not respond to the niclosamide treatment.
indicating an important role for nitroreduction in the bioactivation of niclosamide.

**Aminoniclosamide is mutagenic for nitroreductase-deficient strain YG7132**

As nitroreduction is necessary for the bioactivation of niclosamide, we nitroreduced niclosamide with zinc, and the product of the reaction, aminoniclosamide (Figure 5), was tested in the Ames assay with the liver-S9 fraction. The results showed that the nitroreductase-deficient YG7132 strain significantly increased the average number of revertants (55.44 ± 3.84, n = 18) in response to the treatment with 5–10 μg of aminoniclosamide with the liver-S9 fraction (Wald $t_{19} = 4.18$, $P = 0.000$; Figure 6).

**Discussion**

This study aimed to characterise some aspects of the bioactivation pathway of niclosamide. We hypothesised that CYP and nitroreductase enzymes were determinant factors in this bioactivation. Our results support the idea that a process of nitroreduction by Cnr, followed by an oxidation mediated by CYP1A1, generates mutagenic intermediates from niclosamide.

As expected, the bioactivation of niclosamide depended on the liver-S9 fraction (12). As CYPs are the main phase I enzymes in the liver-S9 fraction (13), it was likely that CYPs might be participating in the bioactivation of niclosamide. Therefore, we used the liver-S9 fraction from rats pretreated with BaP, PB/β-NF or CH, as these compounds preferentially increase the activity of the CYP1A, CYP2B and CYP2E subfamilies, respectively (31,32). These CYP inducers did not differ from one another in their niclosamide mutagenic potential (slope of the dose–response curve), which is consistent with previous studies indicating that the mutagenicity of compounds is rarely determined by the activity of a single P450 enzyme and is instead dependent on several enzymatic pathways (38–40). The finding that the liver-S9 fraction obtained after treatment with either...
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Further experiments with recombinant CYP1A1 and CYP2B1 revealed that the former promoted the highest mutagenic response to niclosamide (Figure 3). The results obtained with the liver-S9 fraction derived from CH-treated rats (Figure 1) and with the CYP2E1 inhibitor DDTC (data not shown) provided evidence that CYP2E1 plays an unimportant role in the bioactivation of niclosamide. The finding that CYP1A1 did play a key role in the mutagenicity of niclosamide agrees with previous studies, which have suggested that CYP1A1 is often involved in the bioactivation of several mutagens and carcinogens (41).

As CYP2B1 affected the mean number of revertants less than CYP1A1 (Figure 3), we focused our attention on confirming the importance of the latter. Accordingly, we used the inhibitor α-NF either with niclosamide or BaP, a positive control with a well-known mutagenic effect (42,43). The use of α-NF reduced equally the number of revertants produced by niclosamide and BaP (Figure 2). Furthermore, the observation that the incubation of niclosamide with the CYP1A1 Supersome™ increased the average number of revertants, which was similar to the results obtained with the liver-S9 fraction, confirmed the participation of CYP1A1 in the bioactivation of niclosamide. By contrast, a greater concentration of the CYP2B1 Supersome™ was required to obtain a slight increase in the number of revertants induced by niclosamide. While PB strongly induces CYP2B1 in rats, the human homologue, CYP2B6, has been only found in small amounts in the liver and does not seem to play an important role in the metabolism of xenobiotics (44).

The liver-S9 fraction protected the bacteria from niclosamide toxicity (Table II), most likely because the main xenobiotic function of CYP metabolism is to prevent toxicity (45). However, after niclosamide was reduced, CYP assumed a mutagenic capacity because of the release of genotoxic metabolite(s) (Figure 4). Chemicals containing nitro groups are known to require the reduction of these nitro groups to the corresponding hydroxylamines to achieve mutagenicity. We suggest that nitroreduction is the rate-limiting step in the bioactivation of niclosamide and that aminoniclosamide might be the reaction product of niclosamide nitroreduction. In addition, the finding that niclosamide interacts with DNA following reductive activation (25) supports the hypothesis that nitroreduction is a critical step in the bioactivation pathway. As we expected, aminoniclosamide became mutagenic in the presence of the liver-S9 fraction, although this compound only caused a slight increase in the number of revertants (Figure 6). This result might be due to differences between in vitro and in situ metabolism in living organisms.

Although a small quantity of niclosamide is absorbed in the gastrointestinal tract (46), results from in vitro and in vivo mutagenicity tests demonstrated that this quantity is sufficient to cause DNA damage (11,12). Niclosamide might be nitroreduced by intestinal bacteria before it enters the bloodstream. This process, in turn, would allow a portion of an administered dose to be absorbed and metabolised by CYP1A1, producing genotoxic metabolites. Such a process could take place in tissues in which CYP1A1 is a constitutive enzyme (47,48) or under conditions in which environmental xenobiotics could induce CYP1A1.

In conclusion, the main finding of our study indicates that the bioactivation of niclosamide relies on CYP1A1 and a nitroreduction mechanism to generate mutagenic metabolites. We cannot rule out the possibility that other enzymes are also involved

Fig. 4. Nitroreductase is a necessary enzyme for the bioactivation of niclosamide. Concentrations of 5 and 7.5 μg/plate of niclosamide were incubated with either YG1021 strain bacteria (overproducing Cnr nitroreductase enzyme) or YG7132 strain bacteria (disrupted Cnr nitroreductase gene). The pre-incubation method was used. 1-Nitropyrene (0.01 μg/plate) was used as positive control; YG1021 (113 revertants/plate), YG7132 (30 revertants/plate). The data are shown as the means ± SEM of 6 trials/treatments. ***P < 0.001 compared with the control condition (0 μg/plate of niclosamide).

Fig. 5. Schematic representation of the formation of aminoniclosamide.

Fig. 6. Cytochrome P450 activates aminoniclosamide in the pre-incubation method. YG7132 strain bacteria (disrupted Cnr nitroreductase gene) were incubated with either YG7132 strain bacteria (disrupted Cnr nitroreductase gene) or YG1021 strain bacteria (overproducing Cnr nitroreductase enzyme) at 5, 7.5 or 10 μg/plate of aminoniclosamide and the liver-S9 fraction from PB/α-NF-treated rats. The data are shown as the means ± SEM of 6 trials/treatments. ***P < 0.001 compared with the control condition (0 μg/plate of aminoniclosamide).
in this process, albeit to a lesser extent; thus, further study would be necessary to explain the precise mechanism of bioactivation.

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References

1. Tesana, S., Thapselpair, P., Thammasari, C., et al. (2012) Effects of bayluscide on Bithynia stainensis geniohalophalus, the first intermediate host of the human liver fluke, Opisthobrachius viverrini, in laboratory and field trials. Parasitol. Int., 61, 52–55.

2. Jones, W. E. (1979) Niclosamide as a treatment for Hymenolepis diminuta and Diphyllidium caninum infection in man. Am. J. Trop. Med. Hyg., 28, 300–302.

3. Dai, J. R., Wang, W., Liang, Y. S., Li, H. J., Guan, X. H. and Zhu, Y. C. (2008) A novel molluscsidal formulation of niclosamide. Parasitol. Res., 103, 405–412.

4. Pearson, R. D. and Hewlett, E. L. (1985) Niclosamide therapy for tapeworm infections. Ann. Intern. Med., 102, 550–551.

5. Wu, C. J., Jan, J. T., Chen, C. M., et al. (2004) Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob Agents Chemother., 48, 2693–2696.

6. Sack, U., Walther, W., Scudiero, D., et al. (2011) Novel effect of antithelminthic Niclosamide on S100A4-mediated metastatic progression in colon cancer. J. Natl. Cancer Inst., 103, 1018–1036.

7. Chen, M., Wang, J., Li, J., Bond, M. C., Ren, X. R., Lyerly, H. K., Barak, L. S. and Chen, W. (2009) The anti-helminthic niclosamide inhibits Wnt/ Frizzled signaling, Biochemistry, 48, 10267–10274.

8. Osada, T., Chen, M., Yang, X. Y., et al. (2011) Antihelminth compound niclosamide downregulates Wnt signaling and elicits antitumor responses in tumors with activating APC mutations. Cancer Res., 71, 4112–4118.

9. Park, S. J., Shin, J. H., Kang, H., Hwang, J. J. and Cho, D. H. (2011) Niclosamide induces mitochondria fragmentation and promotes both apoptotic and autophagic cell death. RMB Rep., 44, 517–522.

10. Vega, S. G., Guzmán, P., García, L., Espinosa, J. and Cortinas de Nava, C. (1988) Sperm shape abnormality and urine mutagenicity in mice treated with niclosamide. Mutat. Res., 204, 269–276.

11. Ostrosky-Wegman, P., García, G., Montero, R., Alvarez Chacón, R. and Cortinas de Nava, C. (1986) Susceptibility to genotoxic effects of niclosamide in human peripheral lymphocytes exposed in vitro and in vivo. Mutat. Res., 173, 81–87.

12. Espinosa-Aguirre, J. J., Reyes, R. E. and Cortinas de Nava, C. (1991) Mutagenic activity of 2-chloro-4-nitroaniline and 5-chlorosalicylic acid in Salmonella typhimurium: two possible metabolites of niclosamide. Mutat. Res., 264, 139–145.

13. Plant, N. (2004) Strategies for using in vitro screens in drug metabolism. Drug Discov. Today, 9, 328–326.

14. Crettol, S., Petrovic, N. and Murray, M. (2010) Pharmacogenetics of phase I and phase II drug metabolism. Curr. Pharm. Des., 16, 204–219.

15. Sun, Y. W., Guengerich, F. P., Sharma, A. K., Boyiri, T., Amin, S. and El-Henawee, M. (2008) Human cytochromes P450 1A1 and 1B1 catalyze ring oxidation but not nitroreduction of environmental pollutant mononitropyrene isomers in primary cultures of human breast cells and cultured MCF-10A and MCF-7 cell lines. Chem. Res. Toxicol., 17, 1077–1085.

16. Roldán, M. D., Pérez-Reinaido, E., Castillo, F. and Moreno-Viviani, C. (2008) Reduction of polynitroaromatic compounds: the bacterial nitroreductases. FEMS Microbiol. Rev., 32, 474–500.

17. Werck-Reichhart, D. and Feyereisen, R. (2000) Cytochromes P450: a success story. Genome Biol., 1, 3003.

18. Buratti, F. M., Volpe, M. T., Menguzzu, A., Vittozzi, L. and Testai, E. (2003) CYP-specific bioactivation of four organophosphate phosphotriesters by human liver microsomes. Toxicol. Appl. Pharmacol., 186, 143–154.

19. Nakajima, T. and Wang, R. S. (1994) Induction of cytochrome P450 by tolune. Int. J. Biochem., 26, 1333–1340.
Human CYP2B6: expression, inducibility and catalytic activities. *Pharmacogenetics*, 9, 295–306.

45. Sonoda, J., Rosenfeld, J. M., Xu, L., Evans, R. M. and Xie, W. (2003) A nuclear receptor-mediated xenobiotic response and its implication in drug metabolism and host protection. *Curr. Drug Metab.*, 4, 59–72.

46. World Health Organization. (2002) *WHO Specifications and Evaluations for Public Health Pesticides* (niclosamide). World Health Organization, Geneva, pp. 1–24.

47. Robie-Suh, K., Robinson, R., Gelboin, H. V. and Guengerich, F. P. (1980) Aryl hydrocarbon hydroxylase is inhibited by antibody to rat liver cytochrome P-450. *Science*, 208, 1031–1033.

48. Shimada, T., Yun, C. H., Yamazaki, H., Gautier, J. C., Beaune, P. H. and Guengerich, F. P. (1992) Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens. *Mol. Pharmacol.*, 41, 856–864.