in the remodeled human IPF lung, both in vascular and in alveolar-interstitial compartments, where they appear to be closely associated with myofibroblasts. This supports the possibility that strategies targeting NOX-4 may be beneficial, not only for treatment of alveolar-interstitial fibrosis but also possibly for the vascular remodeling and pulmonary hypertension that are associated with advanced IPF.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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1. Pache, J.-C. *et al. Nat. Med.* **17**, 31–32 (2011).
2. Patel, N.M., Lederer, D.J., Borczuk, A.C. & Kawut, S.M. *Chest* **132**, 998–1006 (2007).
3. Hecker, L. *et al. Nat. Med.* **15**, 1077–1081 (2009).
4. Shi, Y. *et al. Circulation* **93**, 340–348 (1996).
5. Christen, T. *et al. Circulation* **103**, 882–888 (2001).
6. Frid, M.G. *et al. Am. J. Pathol.* **168**, 659–669 (2006).
7. Haurani, M.J., Cifuentes, M.E., Shepard, A.D. & Pagano, P.J. *Hypertension* **52**, 143–149 (2008).
8. Amara, N. *et al. Thorax* **65**, 733–738 (2010).

Triclosan is minimally effective in rodent malaria models

To the Editor:
The discovery several years ago that *Plasmodium* parasites use a fatty acid synthesis type II pathway (FAS-II), shared by plants and bacteria, raised hopes for the discovery of new antimalarial targets[1] and stimulated the search for FAS-II inhibitors. This pathway is harbored in the *Plasmodium* apicoplast, a nonphotosynthetic plastid of cyanobacterial origin, and is composed of four enzymes. The rate-limiting step is mediated by enoyl acyl carrier protein reductase (FabI)[2]. In mammals, all four enzymatic steps reside in the single multifunctional fatty acid synthesis type I (FAS-I) protein.

The finding that the microbicide triclosan, a chlorinated hydroxyldiphenyl ether, targets lipid synthesis in *Escherichia coli* via inhibition of FabI[3] led to the investigation of its effect on *Plasmodium*. An article published in 2001 in *Nature Medicine*[4] reported that triclosan inhibited *in vitro* propagation of asexual blood stage forms of the human pathogen *Plasmodium falciparum* and cured mice infected with the rodent malarial species *Plasmodium berghei*. This article and a related patent[5] have stimulated a wave of investigations and substantial investments in this agent as a potential new antimalarial drug.

Our groups subsequently confirmed triclosan's *in vitro* activity, yielding half-maximal inhibitory concentration values with *P. falciparum* strains that were comparable to the initial report

Table 1 Results from different laboratories of *the in vivo* efficacy of triclosan in rodent *Plasmodium*–infected mice

Research group	Mouse strain and gender	Mouse malaria strain	Triclosan source, purity standard	Vehicle	Dose (mg per kg body weight per d)	Activity[a] (%, 1 d after treatment)	Cure (%, 30 d after treatment)
Surolia *et al.*[4]	BALB/c[b]	*P. berghei*	Not specified (gift)	Not specified[b]	4 × 3 s.c.	71	0
	Not specified		Not specified		4 × 38 s.c.	100	100
Brun[c]	BALB/c	*P. berghei* ANKA	Ciba (Irgasan),	20% DMSO	4 × 40 s.c.	0	0
	female		USP		4 × 100 s.c.	0	0
					4 × 400 s.c.	0	0
	NMRI/Swiss	*P. berghei* ANKA	Ciba (Irgasan),	20% DMSO	4 × 40 s.c.	0	0
	female		USP		4 × 100 s.c.	0	0
					4 × 400 s.c.	0	0
Fairlamb[c]	NIH/Swiss	*P. berghei* CH15 ANKA	Sigma-Aldrich	98% peanut oil, 2% DMSO	4 × 40 i.p.	0	0
	female		USP				
Croft[c]	TFW	*P. chabaudi* AS	Gift from TDR[d]	Tween 80 and water	4 × 30 s.c.	26	0
	male		Not specified		4 × 30 p.o.	11	
Fidock[c,6]	CD1/Swiss	*P. berghei* ANKA	KIC Chemicals	Peanut oil	3 × 32 s.c.[e]	26	0
	female		USP		3 × 128 s.c.	77	47
					3 × 256 s.c.	52	43
					3 × 512 s.c.	47	29
				0.5% HEC and 0.1% Tween 80	3 × 32 p.o.[e]	28	0
					3 × 128 p.o.	56	27
					3 × 256 p.o.	37	29
					3 × 512 p.o.	40	7

[a]Reduction of infection rate relative to the control group. [b]In a related patent[5], mice were Swiss male and the vehicle used was DMSO (concentration not specified), administered subcutaneously (s.c.). [c]Author of this correspondence. [d]TDR, UNICEF–United Nations Development Programme–World Bank–World Health Organization Special Programme for Research and Training in Tropical Diseases. [e]Daily doses subdivided and administered 6 h apart. Applies to all dose regimens of this experiment. HEC, hydroxyethyl cellulose; i.p., intraperitoneally; p.o., per os (orally).

(**Supplementary Table 1**). However, we all failed to reproduce the *in vivo* data, using multiple *P. berghei* or *Plasmodium chabaudi* mouse models (**Table 1**).

Chemical and genetic validation studies conclusively showed that FabI is not the target for triclosan in blood-stage *P. falciparum* or *P. berghei* parasites and furthermore showed that FabI is not required for growth of *Plasmodium* asexual blood stages[6]. Triclosan's *in vitro* potency was equivalent between wild-type and *fabI*-deficient strains of both malarial species[6]. In mouse studies with *P. berghei* infections, none of us were able to prevent death or observe substantial activity at a daily dose of 38 mg per kg body weight for four consecutive days (noted here as 4 × 38 mg per kg body weight) originally reported as curative[4]. In our studies, *P. berghei*–infected, untreated control mice all showed comparable progressions of parasitemia, leading to death at a similar time as triclosan-treated mice (data not shown). At best, a 77% reduction in parasitemia could be reached at 3 × 128 mg per kg body weight, achieving the same percentage suppression as that originally reported at 4 × 3 mg per kg body weight[4], and a maximum of 47% cure was observed only at 3 × 128 mg per kg body weight (higher doses resulted in lower survival rates, presumably because of toxicity)[6].

The incongruence between our studies and the original report might relate to the purity of the triclosan, to the nature of the vehicle used, to the conditions under which the mice were kept or to gender- or strain-specific activities of the compound. Three of the five triclosan batches referred to in **Table 1** complied with the US Pharmacopeia (USP) specifications, whereas two other batches were gifts of unspecified purity. Hence, differences in the composition of minor components cannot be excluded. Likewise, the use of different vehicles might have contributed to experimental discordance; although not stated in the original report[4], a related patent indicates that DMSO was used[5]. The safety of DMSO via various parenteral routes of administration is well documented (maximum tolerated dose 12.2 g per kg body weight, applied in 50% saline)[7]. However, the possibility of DMSO acting in concert with triclosan cannot be excluded; for example, triclosan administered intraperitoneally (184 mg per kg body weight in 2.5 ml pure DMSO), but not the DMSO-only control, caused a substantial drop in body temperature (10 °C in 3.7 h)[8], which can affect parasite growth. Additionally, because in mice triclosan is primarily excreted in the feces, there is the potential of reabsorption in the gut[9]. Consequently, differences in metabolism may affect the plasma drug concentrations in mice and thus efficacy. Finally, the incongruent findings might also relate to aspects of the experimental design that were not originally reported including the application vehicle, and the application regime, such as subdividing a daily dose into several smaller applications, making it difficult to fully reconcile our findings with the original publication.

Important lessons can be learned from these studies. First, neither genetic nor chemical studies alone can provide sufficiently robust evidence to conclude that an enzyme, a metabolic pathway or a cellular function is a valid drug target[10]. Second, editors and reviewers should insist on full details of animal studies being published either in the main article or in supplementary material. This would reduce unnecessary animal experimentation to confirm major findings. Third, the observed discrepancies in triclosan efficacy highlight the need to include pharmacokinetics in antimalarial drug discovery, an aspect that is all too often lacking in academic institutes engaging in translational research[10].

Note: Supplementary information is available on the Nature Medicine website.

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1. Waller, R.F. *et al. Proc. Natl. Acad. Sci. USA* **95**, 12352–12357 (1998).
2. Ralph, S.A. *et al. Nat. Rev. Microbiol.* **2**, 203–216 (2004).
3. McMurry, L.M. *et al. Nature* **394**, 531–532 (1998).
4. Surolia, N. & Surolia, A. *Nat. Med.* **7**, 167–173 (2001).
5. Surolina, N. *et al.* Use of hydroxydiphenyl ether class of chemicals, as exemplified by triclosan, as an antimalarial and identification of fatty acid synthesis as its target. WO patent 01/00138 A2 (2001).
6. Yu, M. *et al. Cell Host Microbe* **4**, 567–578 (2008).
7. Caujolle, F.M.E. *et al. Ann. N.Y. Acad. Sci.* **141**, 110–126 (1967).
8. Miller, T.L. *et al. J. Toxicol. Environ. Health* **10**, 699–707 (1982).
9. NICNAS. Priority existing chemical draft assessment report triclosan. http://www.nicnas.gov.au/Publications/CAR/PEC/Drafts/Triclosan.asp (2008).
10. Frearson, J.A. *et al. Trends Parasitol.* **23**, 589–595 (2007).

Surolia and Surolia reply:

We previously showed that *Plasmodium falciparum* has the type II fatty acid synthesis (FAS) pathway and outlined the uniqueness of this pathway in terminating at C14 acyl chain. That the pathway shows termination predominantly at C14 acyl chain is an exclusive feature of *Plasmodium falciparum*, and no other organism shows this property. We found that triclosan inhibits FAS with a half-maximal inhibitory concentration of ~800 nM, impeding blood-stage parasitic growth[1]. These findings were validated by several independent groups[2,3]. Targeting of enoyl–acyl carrier protein (ACP) reductase (FabI) by triclosan in malaria was subsequently claimed to be of great therapeutic promise[4].

^{14}C-acetate incorporation in FabI knockouts produces fatty acids containing a C14 acyl chain; therefore, the retention of sensitivity to triclosan by FabI knockouts in red blood cells may be due to enoyl-ACP reductase activity conferred by the presence of other homologs of FabI, for instance FabK, FabL and FabV[1,2,5]. A drug can have multiple targets, and, in time, more than one might emerge for triclosan.

Each of the five enzymes of the fatty acid elongation cycle, together with the ACP, are present during blood stages[2,3]. Also, octanoyl-ACP (an essential precursor for the indispensable cofactor lipoic acid) must be synthesized *de novo* by FAS-II in blood-stage parasites[6]. FAS inhibitors, including triclosan, are effective at lower concentrations on blood-stage parasites as compared to hepatic-stage parasites[7,8], placing uncertainty on the essentiality of FAS at the hepatic stage. Perhaps FAS is required at several parasitic stages.

The effects of an inhibitor may be stage specific and dependent upon factors such as DMSO quality, route of administration, parasite strain, mouse strain and gender, progression of parasitemia, inhibi-