augmented [^{3}H]flunitrazepam binding to GABA-benzodiazepine receptor–Cl^- channel complexes [see M. D. Majewska, N. L. Harrison, R. D. Schwartz, J. L. Barker, S. M. Paul, *Science* **232**, 1004 (1986); N. L. Harrison, M. D. Majewska, J. W. Harrington, J. L. Barker, *J. Pharmacol. Exp. Ther.* **241**, 346 (1987); K. W. Gee, W.-C. Chang, R. E. Brinton, B. S. McEwen, *Eur. J. Pharmacol.* **136**, 419 (1987), A. L. Morrow, P. D. Suzdak, S. M. Paul, *ibid.* **142**, 483 (1987); S. Schwartz-Giblin, M. Canonaco, B. S. McEwen, D. W. Pfaff, *Neuroscience* **25**, 249 (1988)]. By comparison, pregnenolone-sulfate behaved as a $GABA_A$ antagonist [M. D. Majewska and R. D. Schwartz, *Brain Res.* **404**, 355 (1987)].
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27. Consider that, as exemplified in Fig. 2, the slopes of [^{3}H]DAGO binding inhibition isotherms in the presence of the various nonopiate "competitors" were quite similar, that the inhibitory constant (IC_{50}) of 17β-estradiol was equal to that of Ni^{2+} ions, and that the stereospecificity was the opposite of what one would expect if estrogens were of physiological importance to opioid receptors (17α-estradiol was ten times more potent than the natural hormone 17β-estradiol).
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29. This would be of interest because such a hydrophobic pocket (accepting anesthetics or steroids, respectively) on neurotransmitter receptors is hypothetical (*30*) in contrast with allosteric cation binding sites that are typical for a variety of receptors and transmembrane signaling components (*31*). Since there is only anectotal evidence on low-affinity (K_i >100 nM[2]) steroid binding sites on synaptosomal membranes (*32*), the most parsimonial interpretation for the mode of action of "naturally" occurring (≈0.1 to 1 nM) concentrations of steroid hormones in any compartment of the body is the "classical" one: a steroid, owning to its lipophilicity, permeates the cell membrane, subsequently activates high-affinity (K_i ≈0.1 to 1 nM) cytosolic or nuclear receptors and thus ultimately leads to enhanced or silenced transcription via 5′-upstream–located steroid response elements of steroid-responsive genes. Compatible with this mode of action are also all of the known steroid-induced behavioral effects ["changes in mood and psychological parameters" in humans (*1*) and lordosis behavior in rodents as two examples], namely, transcriptional regulation of expression of the various components involved in interneuronal chemical signalling [such as, neurotransmitter receptor molecules, the subunits of the G proteins, the various postreceptor effector units, such as adenylate cyclase, and possibly ion channels as well (*33*)].
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44. We thank I. Gaggl, M. Eichhorn, R. Goerz, R. Gerth, D. Huber, and A. Katki for expert technical assistance and D. Rodbard, B. Cox, L. Werling, and G. Wick for critical discussion of the manuscript.

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Response: Schwarz *et al.* write that psychotomimetic and other behavioral actions of σ opioids, including *d*-*N*-allylnormetazocine (*d*-SKF-10,047), are more likely mediated by phencyclidine (PCP) receptors than by σ receptors. While some behavioral responses to dextrorotatory benzomorphans are mediated through PCP and other receptors, others appear to be linked specifically to σ receptors. For example, rats trained to discriminate *d*-pentazocine show *d*-pentazocine–appropriate responses to other σ ligands in 100% of trials, but they generalize to PCP in only 50% of trials (*1*). In this paradigm, PCP shows about only one-tenth the potency of *d*-SKF-10,047. Activation of A_{10} mesolimbic dopamine neurons by *d*-SKF-10,047 is blocked by rimcazole (*2*), a selective ligand with negligible potency at PCP receptors (*3*). Furthermore, at least seven potential antipsychotic agents that were efficacious in the preclinical tests share the ability to bind with high affinity to σ receptors (*4*). These drugs have negligible affinity at PCP receptors (*4*). Finally, drug-induced locomotor stimulation, which is selectively antagonized by putative σ antagonists such as haloperidol, BMY-14,802, and rimcazole, appears to be mediated through central activation of σ receptors (*5*).

Schwarz *et al.* argue that concentrations of progesterone (extrapolated to be about 8 nM) in cerebrospinal fluid (CSF) would be insufficient for appreciable occupation of σ receptors in the brain. But because progesterone is highly lipid-soluble, it would rapidly enter the brain from the plasma and would most likely show a long half-life in the brain before entering the CSF. Therefore, the CSF concentration of progesterone does not necessarily reflect the "available free" concentration in the brain or other tissues. Likewise, although the "available free" plasma concentration of progesterone in the latter part of human pregnancy is about 50 nM (*6*), which obviously could still occupy about 12% of σ receptors in the peripheral lymphocytes, the progesterone concentration in the placenta could reach approximately 30 μM or even more (*7*). Additionally, high concentrations of progesterone should be present in the ovary and adrenal gland. These results suggest that progesterone may interact with σ receptors on lymphocytes not only systemically but also when blood cells reach organs, such as the placenta, the ovaries, or the adrenal gland, where there is an abundance of progesterone.

Schwarz *et al.* argue against the specificity of progesterone for σ receptors. We contend that progesterone is relatively more specific for σ than for PCP receptors. We did not imply that binding of progesterone to other sites is impossible. Progesterone occupies 50% of σ receptors at a concentration of 268 nM. In figure 2 of Schwarz *et al.*, steroids 17a-E2 and 17b-E2 are shown to "saturate" 50% of the delta opioid receptors, but only at extremely high concentrations (30,000 nM and 100,000 nM, respectively). Although there may be instances in which specific binding is not pharmacologically meaningful, as is the case with the steroids binding to delta receptors, the interaction of progesterone with σ receptors is not comparable. It would be desirable to demonstrate [^{3}H]progesterone binding to σ receptors, but the abundance of nonspecific binding (resulting from the lipophilicity of progesterone) appears to represent a potential problem.

Although glucocorticoids are antiinflammatory, the mechanism of the glucocorticoid-induced immune responses is largely unknown. We agree that the results in the granuloma formation test by Siiteri *et al.* (*7*) might reflect the involvement of glucocorticoid receptors. However, the correlation of the efficacies of preventing granuloma formation with the affinities of steroids at σ receptors is striking. As Schwarz *et al.* and we noted, PCP is immunosuppressive; and it is likely that PCP exerts immunosuppression through σ receptors rather than through PCP receptors. The relative contribution of glucocorticoid and of σ receptors to the

antiinflammatory response to steroids awaits further investigation. Our suggestion that σ receptors may be involved in the antiinflammatory action of certain steroids cannot be ruled out at present. As to the possibility that exogenously administered steroids may induce psychiatric disturbances, several reports have already indicated such a finding (*8*).

We close with the following statement from a review article by Gorski and Gannon (*9*).

> It should be apparent to the reader that a wide variety of mechanisms have been suggested to explain steroid hormone action. One of the minority views mentioned here or perhaps not even referred to in this review may hold the key to understanding steroid hormone action. Until more substantial evidence clearly establishes the validity of one model, it is obviously prudent to maintain an open and critical mind when considering new data and their interpretation.

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Hydrolysis of Carbon Tetrachloride

The ocean water dating technique described by M. Krysell and D. W. R. Wallace (*1*) uses careful measurements of the ratios of concentrations of several anthropogenic halocarbons. Krysell and Wallace recognize that some of these compounds can hydrolyze, but our recent experiments (*2*) show that the standard literature reference for hydrolysis of carbon tetrachloride (CCl_4) (*3*) is wrong. That is, the reaction is reported as second-order in CCl_4, but we have found clear first-order kinetic behavior. It is only for a first-order reaction that the half-life is independent of concentration. In addition, hydrolysis reactions have significant activation energies, so half-lives vary over wide ranges as temperature changes. The 7000-year half-life cited by Krysell and Wallace is a calculated value for CCl_4 assuming a second-order reaction process at 25°C and an initial concentration of 1 ppm. For a first-order reaction, the half-life $\tau = 0.69/k$, where k is the rate constant. Specifically, we have found that, for CCl_4 $k(\text{hyd}) = 4.07 \times 10^{12} \exp(-13{,}790/T)$ min^{-1} and for methyl chloroform (CH_3CCl_3), $k(\text{hyd}) = 2.96 \times 10^{14} \exp(-13{,}970/T)$ min^{-1}, where T is temperature in degrees Kelvin. These rate constant expressions yield the values shown in Table 1:

Table 1.

	CCl_4		CH_3CCl_3	
T (°C)	k (min^{-1})	τ (year)	k (min^{-1})	τ (year)
25	3.25E-8	40.3	1.29E-6	1.0
10	2.80E-9	468	1.08E-7	12.2
0	4.70E-10	2790	1.77E-8	74.2

The results in Table 1 show that Krysell and Wallace correctly assumed negligible degradation of CCl_4 in seawater at 0°C, but for what appears to be the wrong reason. Clearly, CCl_4 does not have a 7000-year half-life under all conditions, but at 0°C is half-life is very long compared with the other ages of interest. Conversely, methylchloroform is also stable enough at 0°C that its concentration should provide valid and independent dating information. However, if this technique is used in significantly warmer water than those found in the Arctic Sea, then the hydrolysis rates of these compounds should be integrated into the age calculations. It should be reemphasized that both CCl_4 and CH_3CCl_3 hydrolyze by (pseudo) first-order reactions with no pH dependence and no significant contribution from other nucleophilic catalytic agents, so that the hydrolysis rate is determined strictly by the temperature.

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Response: The new measurements by Jeffers *et al.*, if applicable to seawater, have implications for the use of CCl_4 as an oceanographic tracer. Figure 1 shows that for a volume fraction of ~92% of oceanic waters, more than 95% of the initial CCl_4 level remains even after 70 years. The absolute amount of CCl_4 loss (not shown) in much of the older, colder water is almost undetectable with the use of current analytical techniques (for example, less than 0.025 pmol/liter). For the relatively small volume fraction of warm ocean waters, corrections for hydrolysis should be applied. Such corrections necessitate that temperature as well as the tracer be modeled. Fortunately the warmer, upper-ocean water masses tend to have sufficiently short renewal times with the ocean surface so that they are labeled with more recently introduced members of the "halocarbon tracer suite" [F-11 (CCl_3F), F-12 (CCl_2F_2), F-113 (CCl_2FCClF_2)]. Hence corrections will be both small and facilitated by supporting tracer data.

The unique CCl_4 input function (*1*) is ideally suited to studies of the circulation of the cold, deep waters that make up the bulk of the world's oceans. Hydrolysis corrections are almost negligible in these waters and in high-latitude seas (for time scales of less than 100 years). Even over 500 years [the average mixing time scale of the ocean deeper than 1500 m (*2*)], losses due to

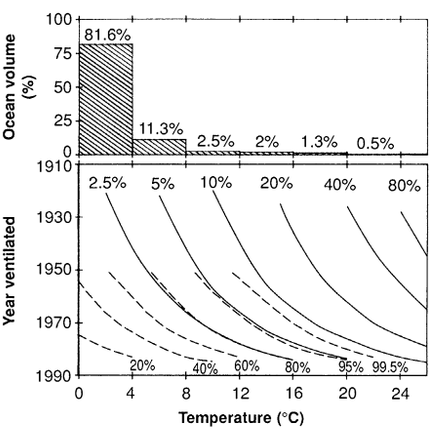

Fig. 1. Plot of the percentage concentration decrease by the year 1990 for CCl_4 (solid lines) and CH_3CCl_3 (dashed lines) for various water temperatures, based on the assumption that a water mass equilibrates with the atmosphere during a "ventilation year" and is subsequently isolated from exchange with the atmosphere and other water masses. Absolute concentration decreases were also calculated; for CCl_4, a preindustrial atmospheric CCl_4 mixing ratio of 6 ppt was assumed (*1*).