POLIO ERADICATION

Efficacy of inactivated poliovirus vaccine in India

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Inactivated poliovirus vaccine (IPV) is efficacious against paralytic disease, but its effect on mucosal immunity is debated. We assessed the efficacy of IPV in boosting mucosal immunity. Participants received IPV, bivalent 1 and 3 oral poliovirus vaccine (bOPV), or no vaccine. A bOPV challenge was administered 4 weeks later, and excretion was assessed 3, 7, and 14 days later. Nine hundred and fifty-four participants completed the study. Any fecal shedding of poliovirus type 1 was 8.8, 9.1, and 13.5% in the IPV group and 14.4, 24.1, and 52.4% in the control group by 6- to 11-month, 5-year, and 10-year groups, respectively (IPV versus control: Fisher's exact test $P < 0.001$). IPV reduced excretion for poliovirus types 1 and 3 between 38.9 and 74.2% and 52.8 and 75.7%, respectively. Thus, IPV in OPV-vaccinated individuals boosts intestinal mucosal immunity.

Since the beginning of polio vaccine development in the 1950s, the choice of which vaccine to use, either the Salk inactivated poliovirus (IPV) or the Sabin live-attenuated oral poliovirus vaccine (OPV), has been fiercely contested (1, 2). The debate continued even after the goal of global polio eradication was established in 1988 (3). Regardless, the global polio eradication initiative (GPEI) chose OPV to eliminate the final chains of poliovirus transmission (4).

Through massive use of OPV, the number of polio-endemic countries decreased from 125 in 1988 to 3 in 2013, and the incidence declined by >99% (5). Wild poliovirus type 2 transmission was interrupted globally in 1999 (6), and wild poliovirus type 3 was last detected in November 2012 (7) (fig. S1). However, parts of three countries (Afghanistan, Nigeria, and Pakistan) continue to report wild poliovirus type 1 (8), and exportation of this virus causes outbreaks (9, 10).

Despite the advantages of OPV (superior mucosal immunity, secondary spread to contacts, ease of administration, and lower price), the vaccine has limitations: low immunogenicity in some tropical countries (11, 12); and incomplete intestinal immunity that wanes

rapidly (13). To interrupt transmission, OPV must be administered to a high proportion of children (1, 4).

The intestinal immunity induced by OPV is incomplete. Even after a full series with OPV, between 10 and 20% of children excrete poliovirus after a challenge with Sabin poliovirus (1, 14). Studies in India demonstrate that a fraction of

children excrete Sabin strains after OPV exposure despite having received more than seven doses of OPV (15). To address the low OPV immunogenicity, the GPEI developed new formulations of OPV, including monovalent OPVs (mOPVs; mOPV1 and mOPV3) in 2005 and bivalent types 1 + 3 OPV (bOPV) in 2009 (16, 17).

Although IPV administered after OPV effectively closes the humoral immunity gaps (2, 18, 19), its effect on intestinal mucosal immunity is less-well characterized. Because mucosal immunity appears to wane rapidly (13), we initiated a trial in Northern India to assess whether IPV could boost mucosal immunity. We enrolled infants aged 6 to 11 months and children aged 5 and 10 years. On enrollment, the three age groups were randomized to receive IPV, bOPV, or no vaccine (control group). Four weeks later, all participants received a challenge dose of bOPV, and poliovirus excretion was measured on days 3, 7, and 14.

Enrollment was completed within 6 days (i.e., during 11 to 16 October 2011), and follow-up visits were concluded by 11 December 2011. Nine hundred and ninety children were enrolled in the trial (fig. S2). Of these, 36 (3.6%) were excluded or withdrawn (1 due to wrong group assignment, 22 with incomplete stool samples, and 13 with incomplete serology samples), leaving 954 (96.4%) for analysis. Virus titer could not be determined for 18 poliovirus type 1 and 13 type 3 stool samples.

There were no significant differences between the groups with regard to median age,

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Table 1. Demographic characteristics and baseline prevalence of poliovirus antibodies ($n = 954$).

Study group/age group	Median age (years) (IQR)*	Gender [% male (95% CI)]	Father's education [% illiterate (95% CI)]
IPV	5.4 (0.9–10.2)	45.9 (40.3–51.6)	45.1 (39.5–50.8)
bOPV	5.5 (0.9–10.2)	47.7 (42.1–53.3)	44.9 (39.3–50.8)
Control	5.5 (0.9–10.2)	49.2 (43.6–54.9)	43.5 (37.9–49.2)
6–11 months	0.8 (0.7–0.9)	45.8 (40.1–51.5)	44.2 (38.5–49.9)
5 years	5.4 (5.2–5.7)	48.8 (43.2–54.3)	44.3 (38.8–49.9)
10 years	10.4 (10.2–10.6)	48.1 (42.5–53.8)	45.0 (39.4–50.6)
Seroprevalence			
	Type 1 % (95% CI)	Type 2 % (95% CI)	Type 3 % (95% CI)
Overall	99.6 (98.9–99.9)	91.6 (89.7–93.3)	93.3 (91.5–94.8)
IPV	99.7 (98.2–100)	92.1 (88.5–94.8)	94.6 (91.5–96.8)
bOPV	99.4 (97.9–99.9)	89.1 (85.2–92.3)	91.0 (87.3–93.9)
Control	99.7 (98.3–100)	93.7 (90.4–96.1)	94.3 (91.2–96.6)
6–11 months	99.0 (97.2–99.8)	81.8 (77.0–86.0)†	89.9 (86.0–93.1)‡
5 years	100 (98.9–100)	95.4 (92.5–97.4)	97.5 (95.2–98.9)
10 years	99.7 (98.3–100)	97.2 (94.7–98.7)	92.2 (88.7–94.9)

*IQR, interquartile range.   †$P < 0.001$ versus 5-year and 10-year age groups.   ‡$P < 0.001$ versus 5-year age group; P value calculated with Fisher's exact (two-sided) test; 95% confidence intervals calculated with Clopper-Pearson method.

gender, father's education, or baseline sero-prevalence levels, which was high (≥99%) for type 1 and somewhat lower for types 2 and 3, particularly in the youngest age group (Table 1).

The prevalence of poliovirus excretion for type 1 and 3 after challenge with bOPV is shown in table S1. For poliovirus type 1, the highest prevalence was 43.8% [95% confidence interval (CI) 34.1–53.8] in 10-year olds in the control group 3 days after challenge compared with 12.5% (95% CI 6.8–20.4) in the IPV group. For poliovirus type 3, the highest prevalence was 44.8% (95% CI 35.0–54.8) 7 days after challenge in the 10-year-olds of the control group compared to 7.7% (95% CI 3.4–14.6) in the IPV group (Fig. 1). Excluding participants excret-ing virus on day 0 (n = 17) produced similar results.

The relative reduction in excretion between IPV group and control group was markedly con-sistent across the poliovirus types. For poliovirus type 1, the decrease in excretion in any stool sam-ple after challenge in the IPV group compared to the control group was 38.9% (8.8% versus 14.4%) in the 6- to 11-month, 62.2% (9.1% versus 24.1%) in the 5-year, and 74.2% (13.5% versus 52.4%) in the 10-year age group; the corresponding decreases for poliovirus type 3 were 71.1% (3.9% versus 13.5%) in the 6- to 11-month, 52.8% (11.8% versus 25.0%) in the 5-year, and 75.7% (12.5% versus 51.4%) in the 10-year age group (table S1).

bOPV significantly decreased excretion of type 1 by 51.9% and type 3 by 40.5% only in the 10-year age group (Fisher's exact test $P < 0.001$ for type 1; $P = 0.002$ for type 3).

For poliovirus type 1, the median duration of excretion was estimated to be 7.6 days (95% CI 6.0–10.1 days) for the IPV group, 8.7 days (95% CI 7.3–10.5) in the control group, and 9.1 days (95% CI 7.4–11.4) in the bOPV group. For poliovirus type 3, the median duration was 10.4 days (95% CI 7.4–15.4) for the IPV group, 13.1 days (95% CI 10.1–17.4) in the control group, and 11.6 days (95% CI 9.0–15.4) in the bOPV group. None of these differences were statistically significant (table S2).

The median virus titers among participants excreting poliovirus type 1 were highest 7 days after challenge, ranging from 4.1 $\log_{10}$ $CCID_{50}$ (50% cell culture infectious dose) per gram of stool (95% CI 3.5–4.7) in the control group to 3.8 $\log10$ $CCID_{50}$ (95% CI 2.1–5.4) in the IPV group, a decrease of 49.9% (Wilcoxon rank-sum test $P = 0.872$). The median titers to poliovirus type 3 were 4.1 $\log_{10}$ $CCID_{50}$ (95% CI 3.6–4.5) in the con-trol group compared to 3.4 $\log_{10}$ $CCID_{50}$ (95% CI 2.6–4.2) in the IPV group, a decrease of 80.0% ($P = 0.143$) (table S3). The relative reduction in the prevalence of excretion in the IPV group com-pared to the control group was between 38.9 and 75.7%, the absolute decrease in median titer of virus in stool specimens was between 0.3 and 0.7 $\log_{10}$ $CCID_{50}$ (at day 7 after challenge), and the absolute decline in length of excretion was between 1.1 and 2.7 days (Fig. 2).

Four weeks after vaccination, an IPV dose induced a humoral immune response (i.e., sero-conversion or fourfold increase in antibody titer) to poliovirus type 1 in 82.9% (95% CI 67.9–92.8), 98.2% (95% CI 90.6–100), and 95.6% (89.1–98.8) of participants in the 6- to 11-month, 5-year, and 10-year age groups, respectively, compared to 2.9% (95% CI 0–15.3), 3.1% (95% CI 0.3–10.8), and 0% (95% CI 0–4.2) in the control group (Fisher's exact test $P < 0.001$ for all age groups). A bOPV dose induced a humoral immune re-sponse in 14.3% (95% CI 4.8–30.3), 12.9% (95% CI 5.7–23.9), and 42.4% (32.1–53.1) of partic-ipants ($P = 0.198$ for the 6- to 11-month group, $P = 0.052$ for the 5-year group, and $P < 0.001$ for 10-year group, compared to control group; significantly lower than the IPV group, $P < 0.001$ for all age groups). Similar levels in hu-moral response were noted against poliovirus type 3 (Table 2).

We divided the antibody titers into quartiles and assessed the excretion prevalence in each quartile. Because the antibody titers were high, we collapsed quartiles 3 and 4 (where the median titers were at the final dilution tested, ≥1:1448). The overall excretion of poliovirus type 1 was 0 (n = 1), 18.2, and 9.9% in quartiles 1, 2, and 3-4, respectively, in the IPV group compared to 40.8, 26.5, and 12.9% in the control group and 30.7, 28.4, and 13.0% in the bOPV group, respective-ly (test for overall trend: Fisher's exact test $P < 0.001$). Similar trends were found for poliovirus type 3.

Our trial provides the following insights: (i) A single dose of IPV boosts intestinal mucosal
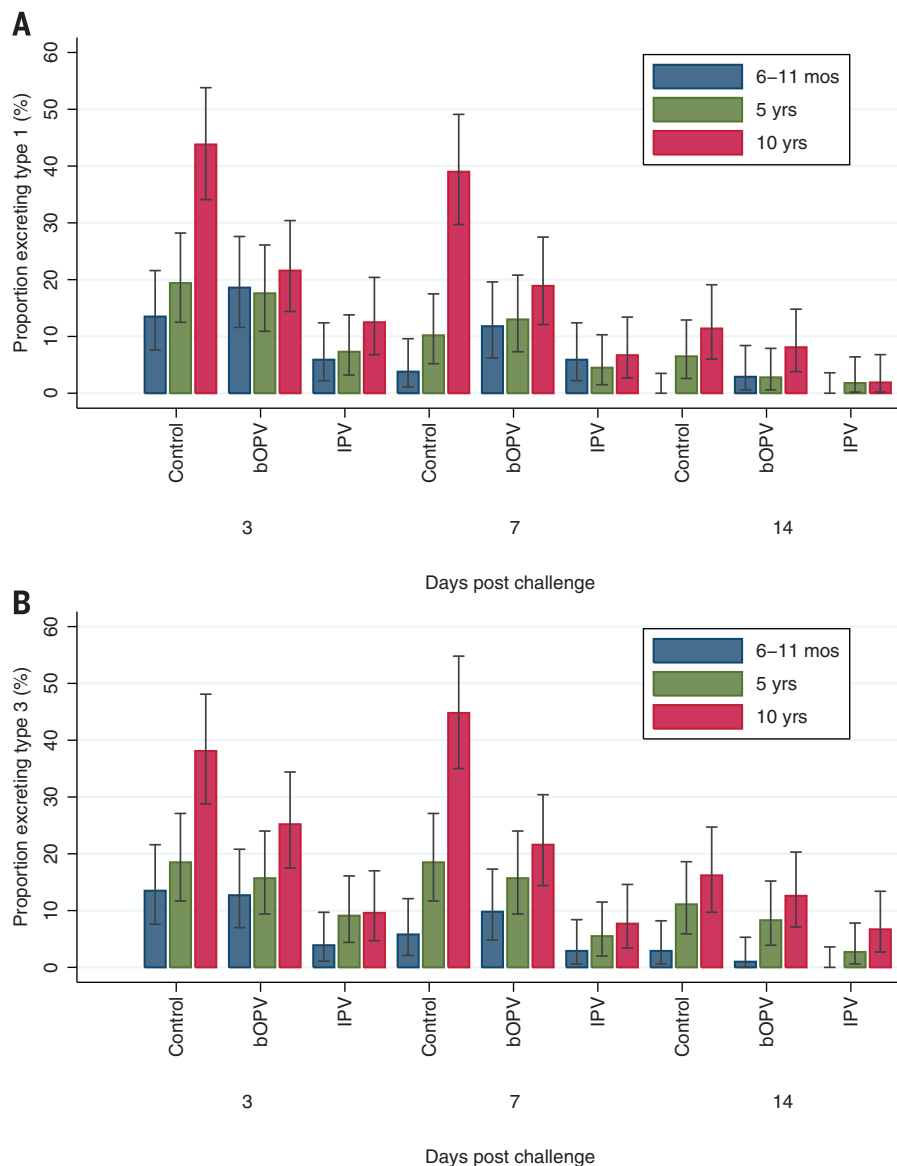
Fig. 1. Excretion of poliovirus type 1 and type 3 after challenge with bOPV. The prevalence of poliovirus type 1 (**A**) and type 3 (**B**) excretions is plotted for each time point after challenge, for each study arm and age group. All subjects (n = 954) are included. Bars represent 95% confidence intervals calculated with the Clopper-Pearson method.

immunity against polioviruses in infants and children with a history of multiple doses of OPV; (ii) the magnitude of this effect is substantially larger after IPV compared to bOPV; and (iii) the oldest age group displayed the highest degree of waning intestinal mucosal immunity manifested by highest prevalence of excretion after challenge.
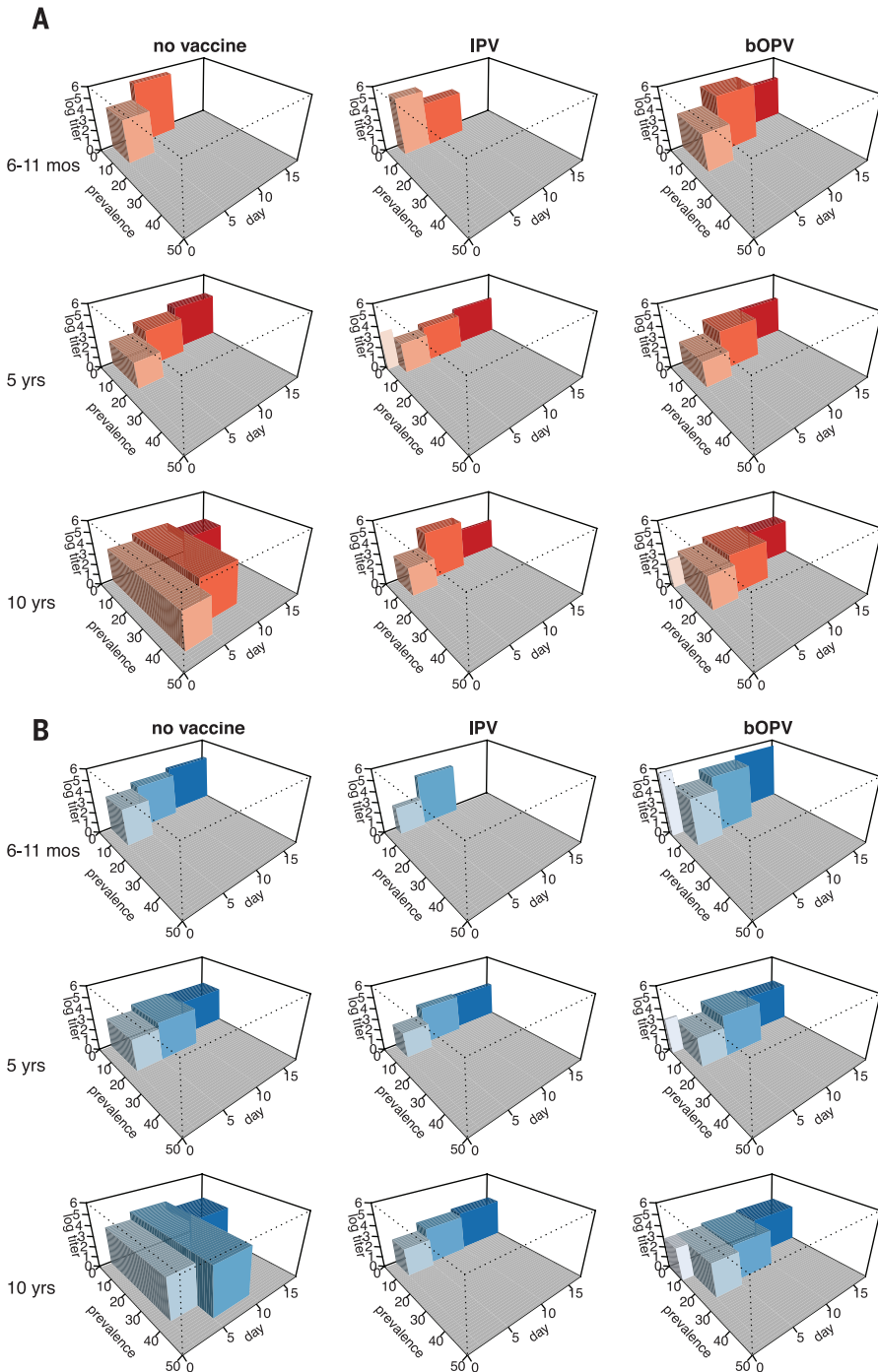

Fig. 2. Magnitude of excretion of poliovirus type 1 and type 3 after bOPV challenge for each study arm and age group. The prevalence of poliovirus type 1 (**A**) and type 3 (**B**) excretion and the mean $\log_{10}$ titer are plotted for each time point after challenge, with the width of the boxes spanning the midpoints between these time points (except for those boxes representing shedding on the day of challenge, where the plot is truncated at day 0). The volume of the boxes therefore roughly represents the total quantity of poliovirus that is excreted (prevalence × quantity × duration). Prevalence measure includes 954 participants. Titer measure includes 303 participants excreting virus after bOPV challenge.

Although IPV reliably induces humoral immunity (18–20) that is highly efficacious in preventing paralytic poliomyelitis (21, 22), the ability of IPV alone to induce intestinal mucosal immunity is limited (23, 24). Studies in countries that do not use OPV show that >90% of IPV-vaccinated children excrete challenge poliovirus (25, 26). Limited data are available on IPV effectiveness in boosting intestinal mucosal immunity in children with a history of multiple doses of OPV (19, 27, 28). Live virus contact to mucosal surfaces appears necessary to induce a specific secretory immunoglobulin A response (29, 30).

The most important comparisons are between the IPV and the control group, where a decrease in the prevalence of excretion of 39 to 76% was found in the IPV group compared to the control group. The decrease after a dose of bOPV was considerably lower, not significant in the 6- to 11-month and 5-year age groups, but 41 to 52% among 10-year-olds.

The study also demonstrated that higher humoral antibody titers are associated with decreased challenge virus excretion, as has been shown previously (4, 19, 27). bOPV increased antibody titers to poliovirus type 2, presumably because of boosting of cross-reacting neutralizing antibodies (31), since we did not isolate poliovirus type 2 from any stool samples. The data support the use of IPV (or OPV) to boost intestinal immunity for travelers to and from polio-infected countries.

Our study had a limitation. We conducted the study in Moradabad district in Uttar Pradesh State of India where the immunogenicity of OPV is low (11, 12). Therefore, extrapolation or generalization of these findings to other areas in India or elsewhere must be done with caution.

The Strategic Advisory Group of Experts (SAGE) recommended in 2012 the introduction of ≥1 dose of IPV in all routine immunization programs for risk mitigation before OPV2 withdrawal (32). A dose of IPV is expected to induce seroconversion or priming in close to 100% of naïve infants (33). Should poliovirus type 2 be reintroduced, a second IPV dose would rapidly boost antibody titers and prevent, or decrease the magnitude of, an outbreak. Furthermore, IPV would be expected to close the remaining type-specific immunity gaps.

Our study provides strong evidence that IPV boosts intestinal immunity among children with a history of multiple OPV doses more effectively than an additional OPV dose. These data provided the scientific foundation for the development of the new polio endgame plan (34) and are guiding the development of strategies to hasten the elimination of the final poliovirus reservoirs and to decrease the vulnerability of polio-free areas to epidemic transmission after poliovirus importations.

Thus, more than 25 years after the World Health Assembly resolution to eradicate poliomyelitis, the answer to the vaccine controversy is

Table 2. Seroconversion or fourfold increase in poliovirus antibody titer between days 0 and 28.

Intervention groups		Type 1			Type 2			Type 3		
		n	% (95% CI)	P value	n	% (95% CI)	P value	n	% (95% CI)	P value
6–11 months	Control	1/34	2.9 (0.0–15.3)	Ref	3/73	4.1 (0.9–11.5)	Ref	3/69	4.3 (0.9–12.2)	Ref
	IPV	34/41	82.9 (67.9–92.8)	<0.001	55/67	82.1 (70.8–90.4)	<0.001	66/72	91.7 (82.7–96.9)	<0.001
	bOPV	5/35	14.3 (4.8–30.3)	0.198	5/74	6.8 (2.2–15.1)	0.719	10/71	14.1 (7.0–24.4)	0.078
5 years	Control	2/64	3.1 (0.3–10.8)	Ref	1/103	1.0 (0.0–5.3)	Ref	2/81	2.5 (0.3–8.6)	Ref
	IPV	56/57	98.2 (90.6–100)	<0.001	90/95	94.7 (88.1–98.3)	<0.001	80/82	97.6 (91.5–99.7)	<0.001
	bOPV	8/62	12.9 (5.7–23.9)	0.052	7/101	6.9 (2.8–13.8)	0.034	14/88	15.9 (9.0–25.2)	0.003
10 years	Control	0/87	0.0 (0.0–4.2)	Ref	2/101	2.0 (0.2–7.0)	Ref	3/102	2.9 (0.6–8.4)	Ref
	IPV	87/91	95.6 (89.1–98.8)	<0.001	99/103	96.1 (90.4–98.9)	<0.001	98/98	100.0 (96.3–100)	<0.001
	bOPV	39/92	42.4 (32.1–53.1)	<0.001	36/105	34.3 (25.3–44.2)	<0.001	54/101	53.5 (43.3–63.5)	<0.001

P values calculated with Fisher's exact (two-tailed) test; 95% confidence intervals calculated with Clopper-Pearson method.

apparent——both vaccines, IPV and OPV, should be used. As a result, the Word Health Organization (WHO) is no longer recommending an all-OPV schedule; rather, it recommends that all OPV-using countries introduce ≥1 dose of IPV into routine vaccine schedules (*35*).

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CARBON CYCLE

Sunlight controls water column processing of carbon in arctic fresh waters

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Carbon in thawing permafrost soils may have global impacts on climate change; however, the factors that control its processing and fate are poorly understood. The dominant fate of dissolved organic carbon (DOC) released from soils to inland waters is either complete oxidation to CO_2 or partial oxidation and river export to oceans. Although both processes are most often attributed to bacterial respiration, we found that photochemical oxidation exceeds rates of respiration and accounts for 70 to 95% of total DOC processed in the water column of arctic lakes and rivers. At the basin scale, photochemical processing of DOC is about one-third of the total CO_2 released from surface waters and is thus an important component of the arctic carbon budget.

Carbon dioxide emissions from inland surface waters to the atmosphere are as large as the net carbon transfer from the atmosphere to Earth's surface (~2 Pg C year^{-1}; 1 Pg = 10^{15} g) (*1–5*). This large flux is affected by the movement of dissolved organic carbon (DOC) from land (*1, 2*) and its subsequent oxidation to CO_2 in fresh waters (*3–5*). The remaining DOC may be unprocessed, flocculated, or partially oxidized and exported in rivers to

Science

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Two vaccines together are better than one alone

Polio is proving difficult to eradicate. Making the choice between administering a live attenuated vaccine orally (Sabin) or an inactivated vaccine (Salk) by injection has been highly controversial. Patients prefer the Sabin vaccine, but it requires many doses to offer immunity. Jafari *et al.* tested the two vaccines together in northern India. The injected vaccine significantly reduced virus shedding and boosted intestinal mucosal immunity in children already given the oral vaccine. Thus, using both vaccines could help speed the eventual global demise of polio.

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